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(54) Title: MODULATORS OF PROTEIN TYROSINE PHOSPHATASES

(57) Abstract

The present invention provides novel compounds, novel compositions, methods of their use, and methods of their manufacture, where such compounds are pharmacologically useful inhibitors of Protein Tyrosine Phosphatases (PTPases, PTPs) such as PTP1B, TC-PTP, CD45, SHP-1, SHP-2, PTPα, PTPε, PTPμ, PTPδ, PTPσ, PTPζ, PTPβ, PTPD1, PTPD2, PTPH1, PTP-MEG1, PTP-LAR, and HePTP. These compounds are indicated in the management or treatment of a broad range of diseases such as autoimmune diseases, acute and chronic inflammation, osteoporosis, various forms of cancer and malignant diseases, and type I diabetes and type II diabetes.

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Modulators of Protein Tyrosine Phosphatases

FIELD OF INVENTION

The present invention provides novel compounds, novel compositions, methods of their use, and methods of their identification, where such compounds are pharmacologically useful inhibitors of Protein Tyrosine Phosphatases (PTPases, PTPs) such as PTP1B, TC-PTP, CD45, SHP-1, SHP-2, PTPα, PTPε, PTPμ, PTPδ, PTPσ, PTPζ, PTPβ, PTPD1, PTPD2, PTPH1, PTP-MEG1, PTP-LAR, and HePTP or ligands of phosphotyrosine units. These compounds are indicated in the management or treatment of a broad range of diseases such as autoimmune diseases, acute and chronic inflammation, osteoporosis, various forms of cancer and malignant diseases, and type I diabetes and type II diabetes.

BACKGROUND OF THE INVENTION

In accordance with the definition of in vivo activity of protein tyrosine phosphatases (PTPases) such as the following non-limiting examples PTPα, LAR, TC-PTP, SHP-1, SHP-2, PTPβ, CD45, PTP1B, HePTP, it has been found that their unique activity plays a major role in the intracellular modulation and regulation of fundamental cellular signalling mechanisms involved in metabolism, growth, proliferation and differentiation (Flint et al., The EMBO J. 12:1937-46,1993; Fischer et al, Science 253:401-6, 1991). Overexpression or altered activity of tyrosine phosphatases can also contribute to the symptoms and progression of various diseases (Wiener, et al., J. Natl. cancer Inst. 86:372-8, 1994; Hunter and Cooper, Ann. Rev. Biochem, 54:897-930, 1985). Furthermore, there is increasing evidence which suggests that inhibition of these PTPases may help treat certain types of diseases such as diabetes, autoimmune disease, acute and chronic inflammation and various forms of cancer.

Protein phosphorylation is now well recognized as an important mechanism utilized by cells to transduce signals during different stages of cellular function (Fischer et al, Science 253:401-6 (1991); Flint et al., The EMBO J. 12:1937-46 (1993)). There are at least two major classes of phosphatases: (1) those that dephosphorylate proteins (or peptides) that contain a phosphate group(s) on a serine or threonine moiety

(termed Ser/Thr phosphatases) and (2) those that remove a phosphate group(s) from the amino acid tyrosine (termed protein tyrosine phosphatases or PTPases).

The PTPases are a family of enzymes that can be classified into two groups: a) intracellular or nontransmembrane PTPases and b) receptor-type or transmembrane PTPases.

Intracellular PTPases: Most known intracellular type PTPases contain a single conserved catalytic phosphatase domain consisting of 220-240 amino acid residues. The regions outside the PTPase domains are believed to play important roles in localizing the intracellular PTPases subcellularly (Mauro, L.J. and Dixon, J.E. *TIBS 19*: 151-155 (1994)). The first intracellular PTPase to be purified and characterized was **PTP1B** which was isolated from human placenta (Tonks *et al., J. Biol. Chem. 263*: 6722-6730 (1988)). Shortly after, PTP1B was cloned (Charbonneau *et al., Proc. Natl. Acad. Sci. USA 86*: 5252-5256 (1989); Chernoff *et al., Proc. Natl. Acad. Sci. USA 87*: 2735-2789 (1989)). Other examples of intracellular PTPases include (1) **T-cell PTPase** (Cool *et al. Proc. Natl. Acad. Sci. USA 86*: 5257-5261 (1989)), (2) **rat brain PTPase** (Guan *et al., Proc. Natl. Acad. Sci. USA 87*:1501-1502 (1990)), (3) neuronal phosphatase **STEP** (Lombroso *et al., Proc. Natl. Acad. Sci. USA 88*: 7242-7246 (1991)), (4) ezrin-domain containing PTPases: **PTPMEG1** (Gu*et al., Proc. Natl. Acad. Sci. USA 88*: 5867-57871 (1991)), **PTPH1** (Yang and Tonks, *Proc. Natl. Acad. Sci. USA 88*: 5949-5953 (1991)), **PTPD1 and PTPD2** (Møller *et al., Proc. Natl. Acad. Sci. USA 91*: 7477-7481 (1994)), **FAP-1/BAS** (Sato *et al., Science 268*: 411-415 (1995); Banville *et al., J. Biol. Chem. 269*: 22320-22327 (1994); Maekawa *et al., FEBS Letters 337*: 200-206 (1994)), and SH2 domain containing PTPases: **PTP1C/SH-PTP1/SHP-1** (Plutzky *et al., Proc. Natl. Acad. Sci. USA 89*: 1123-1127 (1992); Shen *et al., Nature Lond. 352*: 736-739 (1991)) and **PTP1D/Syp/SH-PTP2/SHP-2** (Vogel *et al., Science 259*: 1611-1614 (1993); Feng *et al., Science 259*: 1607-1611 (1993); Bastein *et al., Biochem. Biophys. Res. Comm. 196*: 124-133 (1993)).

Low molecular weight phosphotyrosine-protein phosphatase (**LMW-PTPase**) shows very little sequence identity to the intracellular PTPases described above. However, this enzyme belongs to the PTPase family due to the following characteristics: (i) it possesses the PTPase active site motif: Cys-Xxx-Xxx-Xxx-Xxx-Xxx-Arg (Cirri *et al.*,

Eur. J. Biochem. 214: 647-657 (1993)); (ii) this Cys residue forms a phospho-intermediate during the catalytic reaction similar to the situation with 'classical' PTPases (Cirri *et al., supra*; Chiarugi *et al., FEBS Lett. 310*: 9-12 (1992)); (iii) the overall folding of the molecule shows a surprising degree of similarity to that of PTP1B and *Yersinia* PTP (Su *et al., Nature 370*: 575-578 (1994)).

Receptor-type PTPases consist of a) a putative ligand-binding extracellular domain, b) a transmembrane segment, and c) an intracellular catalytic region. The structures and sizes of the putative ligand-binding extracellular domains of receptor-type PTPases are quite divergent. In contrast, the intracellular catalytic regions of receptor-type PTPases are very homologous to each other and to the intracellular PTPases. Most receptor-type PTPases have two tandemly duplicated catalytic PTPase domains.

The first receptor-type PTPases to be identified were (1) **CD45/LCA** (Ralph, S.J., *EMBO J. 6*: 1251-1257 (1987)) and (2) **LAR** (Streuli *et al., J. Exp. Med. 168*: 1523-1530 (1988)) that were recognized to belong to this class of enzymes based on homology to PTP1B (Charbonneau *et al., Proc. Natl. Acad. Sci. USA 86*: 5252-5256 (1989)). CD45 is a family of high molecular weight glycoproteins and is one of the most abundant leukocyte cell surface glycoproteins and appears to be exclusively expressed upon cells of the hematopoietic system (Trowbridge and Thomas, *Ann. Rev. Immunol. 12*: 85-116 (1994)).

The identification of CD45 and LAR as members of the PTPase family was quickly followed by identification and cloning of several different members of the receptor-type PTPase group. Thus, 5 different PTPases, (3) **PTPα**, (4) **PTPβ**, (5) **PTPδ**, (6) **PTPε**, and (7) **PTPζ**, were identified in one early study (Krueger *et al., EMBO J. 9*: 3241-3252 (1990)). Other examples of receptor-type PTPases include (8) **PTPγ** (Barnea *et al., Mol. Cell. Biol. 13*: 1497-1506 (1995)) which, like **PTPζ** (Krueger and Saito, *Proc. Natl. Acad. Sci. USA 89*: 7417-7421 (1992)) contains a carbonic anhydrase-like domain in the extracellular region, (9) **PTPμ** (Gebbink *et al., FEBS Letters 290*: 123-130 (1991)), (10) **PTPκ** (Jiang *et al., Mol. Cell. Biol. 13*: 2942-2951 (1993)). Based on structural differences the receptor-type PTPases may be classified into subtypes (Fischer *et al., Science 253*: 401-406 (1991)): (I) CD45; (II) LAR, PTPδ, (11) **PTPσ**; (III) PTPβ, (12) **SAP-1** (Matozaki *et al., J. Biol. Chem. 269*: 2075-2081 (1994)), (13)

PTP-U2/GLEPP1 (Seimiya *et al., Oncogene 10*: 1731-1738 (1995); Thomas *et al., J. Biol. Chem. 269*: 19953-19962 (1994)), and (14) **DEP-1**; (IV) PTPα._PTPε. All receptor-type PTPases except Type III contain two PTPase domains. Novel PTPases are continuously identified, and it is anticipated that more than 500 different species will be found in the human genome, i.e. close to the predicted size of the protein tyrosine kinase superfamily (Hanks and Hunter, *FASEB J. 9*: 576-596 (1995)).

PTPases are the biological counterparts to protein tyrosine kinases (PTKs). Therefore, one important function of PTPases is to control, down-regulate, the activity of PTKs. However, a more complex picture of the function of PTPases now emerges. Several studies have shown that some PTPases may actually act as positive mediators of cellular signalling. As an example, the SH2 domain-containing SHP-2 seems to act as a positive mediator in insulin-stimulated Ras activation (Noguchi *et al., Mol. Cell. Biol. 14*: 6674-6682 (1994)) and of growth factor-induced mitogenic signal transduction (Xiao *et al., J. Biol. Chem. 269*: 21244-21248 (1994)), whereas the homologous SHP-1seems to act as a negative regulator of growth factor-stimulated proliferation (Bignon and Siminovitch, *Clin.Immunol. Immunopathol. 73*: 168-179 (1994)). Another example of PTPases as positive regulators has been provided by studies designed to define the activation of the Src-family of tyrosine kinases. In particular, several lines of evidence indicate that CD45 is positively regulating the activation of hematopoietic cells, possibly through dephosphorylation of the C-terminal tyrosine of Fyn and Lck (Chan *et al., Annu. Rev. Immunol. 12*: 555-592 (1994)).

Dual specificity protein tyrosine phosphatases (dsPTPases) define a subclass within the PTPases family that can hydrolyze phosphate from phosphortyrosine as well as from phosphor-serine/threonine. dsPTPases contain the signature sequence of PTPases: Cys-Xxx-Xxx-Xxx--Xxx-Xxx-Arg. At least three dsPTPases have been shown to dephosphorylate and inactivate extracellular signal-regulated kinase (ERKs)/mitogen-activated protein kinase (MAPK): **MAPK phosphatase** (CL100, 3CH134) (Charles *et al., Proc. Natl. Acad. Sci. USA 90*: 5292-5296 (1993)); **PAC-1** (Ward *et al., Nature 367*: 651-654 (1994)); **rVH6** (Mourey *et al., J. Biol. Chem. 271*: 3795-3802 (1996)). Transcription of dsPTPases are induced by different stimuli, e.g. oxidative stress or heat shock (Ishibashi *et al., J. Biol. Chem. 269*: 29897-29902 (1994); Keyse and Emslie, *Nature 359*: 644-647 (1992)). Further, they may be in-

volved in regulation of the cell cycle: **cdc25** (Millar and Russell, *Cell 68*: 407-410 (1992)); **KAP** (Hannon *et al., Proc. Natl. Acad. Sci. USA 91*: 1731-1735 (1994)). Interestingly, tyrosine dephosphorylation of cdc2 by a dual specific phosphatase, cdc25, is required for induction of mitosis in yeast (review by Walton and Dixon, *Annu. Rev. Biochem. 62*: 101-120 (1993)).

PTPases were originally identified and purified from cell and tissue lysates using a variety of artificial substrates and therefore their natural function of dephosphorylation was not well known. Since tyrosine phosphorylation by tyrosine kinases is usually associated with cell proliferation, cell transformation and cell differentiation, it was assumed that PTPases were also associated with these events. This association has now been proven to be the case with many PTPases. PTP1B, a phosphatase whose structure was recently elucidated (Barford et al., Science 263:1397-1404 (1994)) has been shown to be involved in insulin-induced oocyte maturation (Flint et al., The EMBO J. 12:1937-46 (1993)) and recently it has been suggested that the overexpression of this enzyme may be involved in $p185^{c\text{-}erb\ B2}$-associated breast and ovarian cancers (Wiener, et al., J. Natl. cancer Inst. 86:372-8 (1994); Weiner et al., Am. J. Obstet. Gynecol. 170:1177-883 (1994)). The insulin-induced oocyte maturation mechanism has been correlated with the ability of PTP1B to block activation of S6 kinase. The association with cancer is recent evidence which suggests that overexpression of PTP1B is statistically correlated with increased levels of $p185^{c\text{-}erb\ B2}$ in ovarian and breast cancer. The role of PTP1B in the etiology and progression of the disease has not yet been elucidated. Inhibitors of PTP1B may therefore help clarify the role of PTP1B in cancer and in some cases provide therapeutic treatment for certain forms of cancer.

The activity of a number of other newly discussed phosphatases are currently under investigation. Two of these: SHP-1 and Syp/PTP1D/SHPTP2/PTP2C/SHP-2 have recently been implicated in the activation of Platelet Derived Growth Factor and Epidermal Growth Factor induced responses (Li et al., Mole. Cell. Biol. 14:509-17 (1994)). Since both growth factors are involved in normal cell processing as well as disease states such as cancer and arteriosclerosis, it is hypothesized that inhibitors of these phosphatases would also show therapeutic efficacy. Accordingly, the com-

pounds of the present invention, which exhibit inhibitory activity against various PTPases, are indicated in the treatment or management of the foregoing diseases.

PTPases: the insulin receptor signalling pathway/diabetes

Insulin is an important regulator of different metabolic processes and plays a key role in the control of blood glucose. Defects related to its synthesis or signalling lead to diabetes mellitus. Binding of insulin to its receptor causes rapid (auto)phosphorylation of several tyrosine residues in the intracellular part of the b-subunit. Three closely positioned tyrosine residues (the tyrosine-1150 domain) must all be phosphorylated to obtain full activity of the insulin receptor tyrosine kinase (IRTK) which transmits the signal further downstream by tyrosine phosphorylation of other cellular substrates, including insulin receptor substrate-1 (IRS-1) (Wilden *et al., J. Biol. Chem. 267*: 16660-16668 (1992); Myers and White, *Diabetes 42*: 643-650 (1993); Lee and Pilch, *Am. J. Physiol. 266*: C319-C334 (1994); White *et al., J. Biol. Chem. 263*: 2969-2980 (1988)). The structural basis for the function of the tyrosine-triplet has been provided by recent X-ray crystallographic studies of IRTK that showed tyrosine-1150 to be autoinhibitory in its unphosphorylated state (Hubbard *et al., Nature 372*: 746-754 (1994)).

Several studies clearly indicate that the activity of the auto-phosphorylated IRTK can be reversed by dephosphorylation *in vitro* (reviewed in Goldstein, *Receptor 3*: 1-15 (1993); Mooney and Anderson, *J. Biol. Chem. 264*: 6850-6857 (1989)), with the triphosphorylated tyrosine-1150 domain being the most sensitive target for protein-tyrosine phosphatases (PTPases) as compared to the di- and mono- phosphorylated forms (King *et al., Biochem. J. 275*: 413-418 (1991)). It is, therefore, tempting to speculate that this tyrosine-triplet functions as a control switch of IRTK activity. Indeed, the IRTK appears to be tightly regulated by PTP-mediated dephosphorylation *in vivo* (Khan *et al., J. Biol. Chem. 264*: 12931-12940 (1989); Faure *et al., J. Biol. Chem. 267*: 11215-11221 (1992); Rothenberg *et al., J. Biol. Chem. 266*: 8302-8311 (1991)). The intimate coupling of PTPases to the insulin signalling pathway is further evidenced by the finding that insulin differentially regulates PTPase activity in rat hepatoma cells (Meyerovitch *et al., Biochemistry 31*: 10338-10344 (1992)) and in livers from alloxan diabetic rats (Boylan *et al., J. Clin. Invest. 90*: 174-179 (1992)).

Relatively little is known about the identity of the PTPases involved in IRTK regulation. However, the existence of PTPases with activity towards the insulin receptor can be demonstrated as indicated above. Further, when the strong PTPase-inhibitor pervanadate is added to whole cells an almost full insulin response can be obtained in adipocytes (Fantus *et al.*, *Biochemistry 28*: 8864-8871 (1989); Eriksson *et al.*, *Diabetologia 39*: 235-242 (1995)) and skeletal muscle (Leighton *et al.*, *Biochem. J. 276*: 289-292 (1991)). In addition, recent studies show that a new class of peroxovanadium compounds act as potent hypoglycemic compounds *in vivo* (Posner *et al.*,*supra*). Two of these compounds were demonstrated to be more potent inhibitors of dephosphorylation of the insulin receptor than of the EGF-receptor.

It was recently found that the ubiquitously expressed SH2 domain containing PTPase, SHP-2 (Vogel *et al.*, 1993, *supra*), associates with and dephosphorylates IRS-1, but apparently not the IR itself (Kuhné *et al.*, *J. Biol. Chem. 268*: 11479-11481 (1993); (Kuhné *et al.*, *J. Biol. Chem. 269*: 15833-15837 (1994)).

Previous studies suggest that the PTPases responsible for IRTK regulation belong to the class of membrane-associated (Faure *et al.*, *J. Biol. Chem. 267*: 11215-11221 (1992)) and glycosylated molecules (Häring *et al.*, *Biochemistry 23*: 3298-3306 (1984); Sale, *Adv. Prot. Phosphatases 6*: 159-186 (1991)). Hashimoto *et al.* have proposed that LAR might play a role in the physiological regulation of insulin receptors in intact cells (Hashimoto *et al.*, *J. Biol. Chem. 267*: 13811-13814 (1992)). Their conclusion was reached by comparing the rate of dephosphorylation/inactivation of purified IR using recombinant PTP1B as well as the cytoplasmic domains of LAR and PTPa. Antisense inhibition was recently used to study the effect of LAR on insulin signalling in a rat hepatoma cell line (Kulas *et al.*, *J. Biol. Chem. 270*: 2435-2438 (1995)). A suppression of LAR protein levels by about 60 percent was paralleled by an approximately 150 percent increase in insulin-induced auto-phosphorylation. However, only a modest 35 percent increase in IRTK activity was observed, whereas the insulin-dependent phosphatidylinositol 3-kinase (PI 3-kinase) activity was significantly increased by 350 percent. Reduced LAR levels did not alter the basal level of IRTK tyrosine phosphorylation or activity. The authors speculate that LAR could specifically dephosphorylate tyrosine residues that are critical for PI 3-kinase activation either on the insulin receptor itself or on a downstream substrate.

While previous reports indicate a role of PTPα in signal transduction through src activation (Zheng *et al., Nature 359*: 336-339 (1992); den Hertog *et al., EMBO J. 12*: 3789-3798 (1993)) and interaction with GRB-2 (den Hertog *et al., EMBO J. 13*: 3020-3032 (1994); Su *et al., J. Biol. Chem. 269*: 18731-18734 (1994)), a recent study suggests a function for this phosphatase and its close relative PTPε as negative regulators of the insulin receptor signal (Møller *et al.*, 1995 *supra*). This study also indicates that receptor-like PTPases play a significant role in regulating the IRTK, whereas intracellular PTPases seem to have little, if any, activity towards the insulin receptor. While it appears that the target of the negative regulatory activity of PTPases α and ε is the receptor itself, the downmodulating effect of the intracellular TC-PTP seems to be due to a downstream function in the IR-activated signal. Although PTP1B and TC-PTP are closely related, PTP1B had only little influence on the phosphorylation pattern of insulin-treated cells. Both PTPases have distinct structural features that determine their subcellular localization and thereby their access to defined cellular substrates (Frangione *et al., Cell 68*: 545-560 (1992); Faure and Posner, *Glia 9*: 311-314 (1993)). Therefore, the lack of activity of PTP1B and TC-PTP towards the IRTK may, at least in part, be explained by the fact that they do not co-localize with the activated insulin receptor. In support of this view, PTP1B and TC-PTP have been excluded as candidates for the IR-associated PTPases in hepatocytes based on subcellular localization studies (Faure *et al., J. Biol. Chem. 267*: 11215-11221 (1992)).

The transmembrane PTPase CD45, which is believed to be hematopoietic cell-specific, was in a recent study found to negatively regulate the insulin receptor tyrosine kinase in the human multiple myeloma cell line U266 (Kulas et al., J. Biol. Chem. 271: 755-760 (1996)).

Knock-out (K.O.) mice have been useful in elucidating the importance of specific genes in a number of cases. From the results presented above, it would be expected that in particular LAR K.O. mice, PTPα K.O. mice and PTP1B K.O. mice, respectively, could provide important information in relation to insulin signaling. Two groups have generated LAR K.O. mice (Schaapveld at al., Developmental Biology 188: 134-146 (1997); Skarnes et al., Proc.Natl.Acad.Sci.U.S.A. 92:6592-6596 (1995)). Goldstein and coworkers analyzed the LAR K.O. mice from Skarnes et al. (supra) and claimed that these mice exhibited profound defects in glucose-homeostasis and (Ren et al., Diabe-

tes 47:493-497 (1998)). However, the control mice in this study were of a different genetic background than the K.O. mice. Other studies - using the LAR K.O. mice generated by Schaapveld et al. (supra) - did not confirm the results obtained by Ren et al. (supra) (A. R. Sorensen et al., Diabetologia 40 (Suppl 1):556-556 (1997)).

In a recent thorough study, PTP1B K.O. mice (i.e. PTP1B -/- mice) were compared with +/+ and +/- mice of the same genetic background (Elchebly et al., Science 283: 1544-1548 (1999)). In this latter study (Elcheby et al., supra), it was found that disruption of the gene encoding the PTP1B yielded healthy mice that – in the fed state – had blood glucose levels that were slightly lower and concentrations of insulin that were about ½ of those found in their PTP1B+/+ littermates. Further, both insulin and glucose tolerance tests showed enhanced insulin sensitivity in the PTP K.O. mice. On a high-fat diet, PTP1B-/- and PTP1B-/+ mice were resistant to weight gain and remained insulin sensitive – in contrast to the PTP1B +/+ mice that rapidly gained weight and became insulin resistant. Analysis of the levels of tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1) showed increased phosphorylation of these proteins in PTP1B -/- mice (liver and muscle) in comparison with the PTP1B +/+ mice. All of these findings are in accordance with the notion that PTP1B is likely to play a major role as negative regulator of the insulin receptor signaling pathway - and in contrast to the above-mentioned in vitro studies. The authors conclude that 'these results make PTP-1B a potential therapeutic target for treatment of type 2 diabetes and obesity' (Elcheby et al., supra).

PTPases: somatostatin

Somatostatin inhibits several biological functions including cellular proliferation (Lamberts *et al., Molec. Endocrinol. 8*: 1289-1297 (1994)). While part of the antiproliferative activities of somatostatin are secondary to its inhibition of hormone and growth factor secretion (e.g. growth hormone and epidermal growth factor), other antiproliferative effects of somatostatin are due to a direct effect on the target cells. As an example, somatostatin analogs inhibit the growth of pancreatic cancer presumably via stimulation of a single PTPase, or a subset of PTPases, rather than a general activation of PTPase levels in the cells (Liebow *et al., Proc. Natl. Acad. Sci. USA 86*: 2003-2007 (1989); Colas *et al., Eur. J. Biochem. 207*: 1017-1024 (1992)). In a recent study it was found that somatostatin stimulation of somatostatin receptors SSTR1, but not

SSTR2, stably expressed in CHO-K1 cells can stimulate PTPase activity and that this stimulation is pertussis toxin-sensitive. Whether the inhibitory effect of somatostatin on hormone and growth factor secretion is caused by a similar stimulation of PTPase activity in hormone producing cells remains to be determined.

PTPases: the immune system/autoimmunity

Several studies suggest that the receptor-type PTPase CD45 plays a critical role not only for initiation of T cell activation, but also for maintaining the T cell receptor-mediated signalling cascade. These studies are reviewed in: (Weiss A., *Ann. Rev. Genet. 25*: 487-510 (1991); Chan *et al., Annu. Rev. Immunol. 12*: 555-592 (1994); Trowbridge and Thomas, *Annu. Rev. Immunol. 12*: 85-116 (1994)).

CD45 is one of the most abundant of the cell surface glycoproteins and is expressed exclusively on hemopoetic cells. In T cells, it has been shown that CD45 is one of the critical components of the signal transduction machinery of lymphocytes. In particular, evidence has suggested that CD45 phosphatase plays a pivotal role in antigen-stimulated proliferation of T lymphocytes after an antigen has bound to the T cell receptor (Trowbridge, *Ann. Rev. Immunol*, 12:85-116 (1994)). Several studies suggest that the PTPase activity of CD45 plays a role in the activation of Lck, a lymphocyte-specific member of the Src family protein-tyrosine kinase (Mustelin etal., Proc. Natl. Acad. Sci. USA 86: 6302-6306 (1989); Ostergaard et al., Proc. Natl. Acad. Sci. USA 86: 8959-8963 (1989)). These authors hypothesized that the phosphatase activity of CD45 activates Lck by dephosphorylation of a C-terminal tyrosine residue, which may, in turn, be related to T-cell activation. In a recent study it was found that recombinant p56lck specifically associates with recombinant CD45 cytoplasmic domain protein, but not to the cytoplasmic domain of the related PTPαa (Ng et al., *J. Biol. Chem.* 271: 1295-1300 (1996)). The p56lck-CD45 interaction seems to be mediated via a nonconventional SH2 domain interaction not requiring phosphotyrosine. In immature B cells, another member of the Src family protein-tyrosine kinases, Fyn, seems to be a selective substrate for CD45 compared to Lck and Syk (Katagiri et al., *J. Biol. Chem.* 270: 27987-27990 (1995)).

Studies using transgenic mice with a mutation for the CD45-exon6 exhibited lacked mature T cells. These mice did not respond to an antigenic challenge with the typi-

cal T cell mediated response (Kishihara *et* al., *Cell* 74:143-56 (1993)). Inhibitors of CD45 phosphatase would therefore be very effective therapeutic agents in conditions that are associated with autoimmune disease.

CD45 has also been shown to be essential for the antibody mediated degranulation of mast cells (Berger *et* al., *J. Exp. Med.* 180:471-6 (1994)). These studies were also done with mice that were CD45-deficient. In this case, an IgE-mediated degranulation was demonstrated in wild type but not CD45-deficient T cells from mice. These data suggest that CD45 inhibitors could also play a role in the symptomatic or therapeutic treatment of allergic disorders.

Another recently discovered PTPase, an inducible lymphoid-specific protein tyrosine phosphatase (HePTP) has also been implicated in the immune response. This phosphatase is expressed in both resting T and B lymphocytes, but not non-hemopoetic cells. Upon stimulation of these cells, mRNA levels from the HePTP gene increase 10-15 fold (Zanke *et al.*, *Eur. J. Immunol.* 22:235-239 (1992)). In both T and B cells HePTP may function during sustained stimulation to modulate the immune response through dephosphorylation of specific residues. Its exact role, however remains to be defined.

Likewise, the hematopoietic cell specific SHP-1 seems to act as a negative regulator and play an essential role in hematopoietic cell development. Thus, SHP-1 plays a significant role in regulating the erythropoietin signalling pathway, which is enhanced in mice lacking intact SHP-1 (Schultz et al. *Cell* 73: 1445-1454. In accordance with the above-mentioned important function of CD45, HePTP and SHP-1, selective PTPase inhibitors may be attractive drug candidates both as immunosuppressors and as immunostimulants as well as inhibitors and stimulants of the hematopoietic system. One recent study illustrates the potential of PTPase inhibitors as immunmodulators by demonstrating the capacity of the vanadium-based PTPase inhibitor, BMLOV, to induce apparent B cell selective apoptosis compared to T cells (Schieven et al., *J. Biol. Chem.* 270: 20824-20831 (1995)).

PTPases: cell-cell interactions/cancer

Focal adhesion plaques, an *in vitro* phenomenon in which specific contact points are formed when fibroblasts grow on appropriate substrates, seem to mimic, at least in part, cells and their natural surroundings. Several focal adhesion proteins are phosphorylated on tyrosine residues when fibroblasts adhere to and spread on extracellular matrix (Gumbiner, *Neuron 11*, 551-564 (1993)). However, aberrant tyrosine phosphorylation of these proteins can lead to cellular transformation. The intimate association between PTPases and focal adhesions is supported by the finding of several intracellular PTPases with ezrin-like N-terminal domains, e.g. PTPMEG1 (Gu *et al., Proc. Natl. Acad. Sci. USA 88*: 5867-5871 (1991)), PTPH1 (Yang and Tonks, *Proc. Natl. Acad. Sci. USA 88*: 5949-5953 (1991)) and PTPD1 (Møller *et al., Proc. Natl. Acad. Sci. USA 91*: 7477-7481 (1994)). The ezrin-like domain show similarity to several proteins that are believed to act as links between the cell membrane and the cytoskeleton. PTPD1 was found to be phosphorylated by and associated with c-src *in vitro* and is hypothesized to be involved in the regulation of phosphorylation of focal adhesions (Møller *et al.*, *supra*).

PTPases may oppose the action of tyrosine kinases, including those responsible for phosphorylation of focal adhesion proteins, and may therefore function as natural inhibitors of transformation. TC-PTP, and especially the truncated form of this enzyme (Cool *et al., Proc. Natl. Acad. Sci. USA 87:* 7280-7284 (1990)), can inhibit the transforming activity of v-*erb* and v-*fms* (Lammers *et al., J. Biol. Chem. 268:* 22456-22462 (1993); Zander *et al., Oncogene 8:* 1175-1182 (1993)). Moreover, it was found that transformation by the oncogenic form of the *HER2/neu* gene was suppressed in NIH 3T3 fribroblasts overexpressing PTP1B (Brown-Shimer *et al., Cancer Res. 52:* 478-482 (1992)).

The expression level of PTP1B was found to be increased in a mammary cell line transformed with *neu* (Zhay *et al., Cancer Res. 53:* 2272-2278 (1993)). The intimate relationship between tyrosine kinases and PTPases in the development of cancer is further evidenced by the recent finding that PTPε is highly expressed in murine mammary tumors in transgenic mice over-expressing c-*neu* and v-Ha-*ras*, but not c-*myc* or *int-2* (Elson and Leder, *J. Biol. Chem. 270:* 26116-26122 (1995)). Further, the human

gene encoding PTPg was mapped to 3p21, a chromosomal region which is frequently deleted in renal and lung carcinomas (LaForgia *et al., Proc. Natl. Acad. Sci. USA 88*: 5036-5040 (1991)).

In this context, it seems significant that PTPases appear to be involved in controlling the growth of fibroblasts. In a recent study it was found that Swiss 3T3 cells harvested at high density contain a membrane-associated PTPase whose activity on an average is 8-fold higher than that of cells harvested at low or medium density (Pallen and Tong, *Proc. Natl. Acad. Sci. USA 88*: 6996-7000 (1991)). It was hypothesized by the authors that density-dependent inhibition of cell growth involves the regulated elevation of the activity of the PTPase(s) in question. In accordance with this view, a novel membrane-bound, receptor-type PTPase, DEP-1, showed enhanced (>=10-fold) expression levels with increasing cell density of WI-38 human embryonic lung fibroblasts and in the AG1518 fibroblast cell line (Östman *et al., Proc. Natl. Acad. Sci. USA 91*: 9680-9684 (1994)).

Two closely related receptor-type PTPases, PTPκ and PTPμ, can mediate homophilic cell-cell interaction when expressed in non-adherent insect cells, suggesting that these PTPases might have a normal physiological function in cell-to-cell signalling (Gebbink *et al., J. Biol. Chem. 268*: 16101-16104 (1993); Brady-Kalnay *et al., J. Cell Biol. 122*: 961-972 (1993); Sap *et al., Mol. Cell. Biol. 14*: 1-9 (1994)). Interestingly, PTPk and PTPμ do not interact with each other, despite their structural similarity (Zondag *et al., J. Biol. Chem. 270*: 14247-14250 (1995)). From the studies described above it is apparent that PTPases may play an important role in regulating normal cell growth. However, as pointed out above, recent studies indicate that PTPases may also function as positive mediators of intracellular signalling and thereby induce or enhance mitogenic responses. Increased activity of certain PTPases might therefore result in cellular transformation and tumor formation. Indeed, in one study overexpression of PTPα was found to lead to transformation of rat embryo fibroblasts (Zheng, *supra*). In addition, a novel PTP, SAP-1, was found to be highly expressed in pancreatic and colorectal cancer cells. SAP-1 is mapped to chromosome 19 region q13.4 and might be related to carcinoembryonic antigen mapped to 19q13.2 (Uchida *et al., J. Biol. Chem. 269*: 12220-12228 (1994)). Further, the dsPTPase, cdc25, dephosphorylates cdc2 at Thr14/Tyr-15 and thereby functions as positive regulator of

mitosis (reviewed by Hunter, *Cell 80*: 225-236 (1995)). Inhibitors of specific PTPases are therefore likely to be of significant therapeutic value in the treatment of certain forms of cancer.

PTPases: platelet aggregation

Recent studies indicate that PTPases are centrally involved in platelet aggregation. Agonist-induced platelet activation results in calpain-catalyzed cleavage of PTP1B with a concomitant 2-fold stimulation of PTPase activity (Frangioni *et al.*, *EMBO J. 12:* 4843-4856 (1993)). The cleavage of PTP1B leads to subcellular relocation of the enzyme and correlates with the transition from reversible to irreversible platelet aggregation in platelet-rich plasma. In addition, the SH2 domain containing PTPase, SHP-1/SH-PTP1, was found to translocate to the cytoskeleton in platelets after thrombin stimulation in an aggregation-dependent manner (Li *et al.*, *FEBS Lett. 343:* 89-93 (1994)).

Although some details in the above two studies were recently questioned there is over-all agreement that PTP1B and SHP-1 play significant functional roles in platelet aggregation (Ezumi *et al.*, *J. Biol. Chem. 270:* 11927-11934 (1995)). In accordance with these observations, treatment of platelets with the PTPase inhibitor pervanadate leads to significant increase in tyrosine phosphorylation, secretion and aggregation (Pumiglia *et al.*, *Biochem. J. 286:* 441-449 (1992)).

PTPases: osteoporosis

The rate of bone formation is determined by the number and the activity of osteoblasts, which in term are determined by the rate of proliferation and differentiation of osteoblast progenitor cells, respectively. Histomorphometric studies indicate that the osteoblast number is the primary determinant of the rate of bone formation in humans (Gruber *et al.*, *Mineral Electrolyte Metab. 12*: 246-254 (1987); reviewed in Lau *et al.*, *Biochem. J. 257*: 23-36 (1989)). Acid phosphatases/PTPases may be involved in negative regulation of osteoblast proliferation. Thus, fluoride, which has phosphatase inhibitory activity, has been found to increase spinal bone density in osteoporotics by increasing osteoblast proliferation (Lau *et al.*, *supra*). Consistent with this ob-

servation, an osteoblastic acid phosphatase with PTPase activity was found to be highly sensitive to mitogenic concentrations of fluoride (Lau *et al., J. Biol. Chem. 260*: 4653-4660 (1985); Lau *et al., J. Biol. Chem. 262*: 1389-1397 (1987); Lau *et al., Adv. Protein Phosphatases 4*: 165-198 (1987)). Interestingly, it was recently found that the level of membrane-bound PTPase activity was increased dramatically when the osteoblast-like cell line UMR 106.06 was grown on collagen type-I matrix compared to uncoated tissue culture plates. Since a significant increase in PTPase activity was observed in density-dependent growth arrested fibroblasts (Pallen and Tong, *Proc. Natl. Acad. Sci. 88*: 6996-7000 (1991)), it might be speculated that the increased PTPase activity directly inhibits cell growth. The mitogenic action of fluoride and other phosphatase inhibitors (molybdate and vanadate) may thus be explained by their inhibition of acid phosphatases/PTPases that negatively regulate the cell proliferation of osteoblasts. The complex nature of the involvement of PTPases in bone formation is further suggested by the recent identification of a novel parathyroid regulated, receptor-like PTPase, OST-PTP, expressed in bone and testis (Mauro *et al., J. Biol. Chem. 269*: 30659-30667 (1994)). OST-PTP is up-regulated following differentiation and matrix formation of primary osteoblasts and subsequently down-regulated in the osteoblasts which are actively mineralizing bone in culture. It may be hypothesized that PTPase inhibitors may prevent differentiation via inhibition of OST-PTP or other PTPases thereby leading to continued proliferation. This would be in agreement with the above-mentioned effects of fluoride and the observation that the tyrosine phosphatase inhibitor orthovanadate appears to enhance osteoblast proliferation and matrix formation (Lau *et al., Endocrinology 116*: 2463-2468 (1988)). In addition, it was recently observed that vanadate, vanadyl and pervanadate all increased the growth of the osteoblast-like cell line UMR106. Vanadyl and pervanadate were stronger stimulators of cell growth than vanadate. Only vanadate was able to regulate the cell differentiation as measured by cell alkaline phosphatase activity (Cortizo *et al., Mol. Cell. Biochem. 145*: 97-102 (1995)).

PTPases: microorganisms

Dixon and coworkers have called attention to the fact that PTPases may be a key element in the pathogenic properties of *Yersinia* (reviewed in Clemens *et al. Molecular Microbiology 5*: 2617-2620 (1991)). This finding was rather surprising since tyrosine

phosphate is thought to be absent in bacteria. The genus *Yersinia* comprises 3 species: *Y. pestis* (responsible for the bubonic plague), *Y. pseudoturberculosis* and *Y. enterocolitica* (causing enteritis and mesenteric lymphadenitis). Interestingly, a dual-specificity phosphatase, VH1, has been identified in Vaccinia virus (Guan *et al., Nature 350*: 359-263 (1991)). These observations indicate that PTPases may play critical roles in microbial and parasitic infections, and they further point to PTPase inhibitors as a novel, putative treatment principle of infectious diseases.

BRIEF DESCRIPTION OF THE INVENTION

As described above, PTPases are essential elements in a variety of cellular signalling processes. Inhibitors or modulators of these enzymes, or a given subset of PTPases, or even one specific PTPase, are therefore attractive drug candidates. However, until now only a limited set of inhibitors has been reported in the literature. Some of the most potent inhibitors are analogs of tyrosine-phosphorylated peptides and therefore not suitable candidates for oral use.

I. Vanadate and Pervanadate. Vanadate and pervanadate/peroxovanadium compounds induce insulin-like effects in cells and animals. A few anecdotal, clinical studies with formulas of vanadate have shown positive effects in humans with type II diabetes. The mechanism of action at the cellular level is believed to be via inhibition of PTPases. Pervanadate (the complexes of vanadate and hydrogen peroxide) were recently found to be an irreversible inhibitor of PTPases via oxidation of the active site catalytic cysteine (Huyer et al., J. Biol. Chem. 272: 843-851 (1997)). Further, the effects are very sensitive to the assay constituents such as EDTA and reducing agents (e.g. dithiothreitol, DTT). It should be noted that vanadate and peroxovanadium-based compounds inhibit a broad range of PTPases. It conceivable that the mechanism of action, i.e. oxidation of the active site cysteine, will cause substantial problems when attempting to develop compounds that selectively inhibit specific PTPases. Further, the toxic effects of vanadate, pervanadate and peroxovanadium-based inhibitors will likely prevent their use for treatment of chronic diseases such as diabetes.

II. Bisphosphonates. Bisphosphonates have been successfully used as therapeutic agents for treatment of bone disorders such as osteoporosis and Paget's disease. Bisphosphonates inhibits osteoclast resorption which results in reduced bone turn-over and a net gain in bone mineral density (for review, see Rodan and Fleisch, J. Clin. Invest. 97: 2692-2696 (1996)). It is currently believed that the mechanism of action at the cellular level is via bisphosphonates' inhibitory activity against PTPases (in the osteoclasts) (Skorey et al., J. Biol. Chem. 272: 22472-22480 (1997); Opas et al., Biochemical Pharmacology 54: 721-727 (1997)). However, the inhibitory effect of alendronate was found to be very sensitive to assay constituents such as EDTA and DTT). Further, it was shown that the inhibition is time dependent. The mechanism of action at the biochemical level was recently shown to be via oxidation of the catalytic cysteine in the active site (Skorey et al., *vide supra*). It should be noted that bisphosphonates inhibit a broad range of PTPases. It is conceivable that the mechanism of action, i.e. oxidation of the active site cysteine, will cause substantial problems when attempting to develop bisphosphonate-based compounds that selectively inhibit specific PTPases.

III. Gold compounds. it was recently shown that disodium aurothiomalate (AuTM), which been successfully used in the treatment of autoimmune and inflammatory disorders, act as an inhibitor of PTPases (Wang et al, Biochemical Pharmacology 54:703-711 (1997)). However, AuTM appears to inhibit PTPases via its interaction with the active site nucleophilic cysteine in these enzymes. Dithiothreitol can prevent or almost completely prevent this inhibition, which is in contrast to compounds of the present invention. As for the bisphosphonates, substantial problems are likely to arise if the gold compounds were to be used to develop selective inhibitors.

The inhibitors described above are non-selective. Some of the observed toxic effects or side effects are likely to be caused, at least in part, by their lack of selectivity.

Thus, there is a strong need for a non-peptide, general, competitive or mixed-type, reversible classical PTPase inhibitor or compounds, which can be used for further optimization to potent and selective inhibitors.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1. Steady State Enzyme Kinetic Analysis. PTP1B was incubated in 96 well plates with different concentrations of the substrate, para-nitrophenyl phosphate (pNPP), and the inhibitor, 2-(oxalylamino)benzoic acid: 0, 7.4, 22.2, 66.7 and 200 μM - final assay concentration). Buffer: 100 mM sodium acetate pH 5.5, 50 mM NaCl, 5 mM dithiothreitol, 0.1 % (w/v) bovine serum albumin. Incubation time: 45 minutes; temperature: 25°C. Sodium hydroxide was added and the absorbance read at 405 nm. (**A**) Michaelis Menten Plots; (**B**) plot of the apparent Km values relative to the concentration of the inhibitor; (**C**) plot of the apparent Vmax relative to the concentration of the inhibitor. For further details, see the section DEFINITIONS. Exp.no. 1230-5.

Figure 2. Steady State Enzyme Kinetic Analysis. Conditions as in Figure 2, except that the buffer was (final assay concentration): 100 mM sodium acetate pH 5.5, 50 mM NaCl, 5 mM glutathione, 1 mM EDTA, and 0.1 % bovine serum albumin. Incubation time: 60 mins. Exp. 1167-3

Figure 3. Time course experiments. (A) PTP1B was incubated in 96 well plates at room temperature with 2.5 mM para-nitrophenol phosphate (pNPP) in a buffer containing. The compound, 2-(oxalylamino)benzoic acid, was added at final assay concentrations of 250, 125 and 62.5 μM. The reaction was started by addition of the enzyme, and the stopped at the indicated time intervals by addition of NaOH. The absorbance at 405 nm was finally measured in all wells. **(B)** As in (A), except that EDTA was added to a final concentration of 1 mM.

Figure 4. Steady State Enzyme Kinetic Analysis. PTP1B was incubated in 96 well plates with different concentrations of the substrate, para-nitrophenyl phosphate, and the inhibitor, 3-(oxalyl-amino)naphthalene-2-carboxylic acid : 0, 3.7, 11.1, 33.3 and 100 μM - final assay concentration). Buffer (final assay concentration): 100 mM sodium acetate pH 5.5, 50 mM NaCl, 5 mM glutathione, 1 mM EDTA, and 0.1 % bovine serum albumin. Incubation time:60 mins.; temperature: 25 °C. Sodium hydroxide was added and the absorbance read at 405 nm. (**A**) Michaelis Menten Plots; (**B**) plot of the apparent Km values relative to the concentration of the inhibitor; (**C**) plot of the

apparent Vmax relative to the concentration of the inhibitor. For further details, see the section DEFINITIONS.

Figure 5. Steady State Enzyme Kinetic Analysis. PTP1B was incubated in 96 well plates with different concentrations of the substrate, para-nitrophenyl phosphate, and the inhibitor, 2-(oxalyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid: 0, 18.5, 55.6, 166.7 and 500 μM - final assay concentration). Buffer (final assay concentration): 100 mM sodium acetate pH 5.5, 50 mM NaCl, 5 mM dithiothreitol, 0.1 % (w/v) bovine serum albumin. Incubation time:60 mins.; temperature: 25 °C. Sodium hydroxide was added and the absorbance read at 405 nm. (**A**) Michaelis Menten Plots; (**B**) plot of the apparent Km values relative to the concentration of the inhibitor; (**C**) plot of the apparent Vmax relative to the concentration of the inhibitor. For further details, see the section DEFINITIONS.

Figure 6. Steady State Enzyme Kinetic Analysis. (A) Michaelis Menten Plots. PTPα was incubated in 96 well plates with different concentrations of the substrate, para-nitrophenyl phosphate (pNPP), and the inhibitor, 5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid: 0, 7.4, 22.2, 66.7 and 200 μM - final assay concentrations. Buffer (final assay concentration): 100 mM sodium acetate pH 5.5, 50 mM NaCl, 5 mM glutathione, 1 mM EDTA, and 0.1 % bovine serum albumin. Reaction temperature: 25 °C. After 60 minutes, 10 μl of a 0.5 M sodium hydroxide solution (in 50 percent (vol/vol) ethanol) was added to each well and the absorbance was read at 405 nm. (B) Michaelis Menten Plots. PTPα was incubated in 96 well plates with different concentrations of the substrate, para-nitrophenyl phosphate, and the inhibitor, 5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid: 0, 37, 111.1, 333.3 and 1000 μM - final assay concentrations. Buffer (final assay concentration): 50 mM HEPES pH 7.0, 100 mM NaCl, 5 mM glutathione, 1 mM EDTA, and 0.1 % bovine serum albumin. Reaction temperature: 25 °C. After 60 minutes, 20 μl of a 0.5 M sodium hydroxide solution (in 50 percent (vol/vol) ethanol) was added to each well and the absorbance was read at 405 nm. For further details, see the section DEFINITIONS.

Figure 7. Homology Tree Based on Multiple Sequence Alignments of PTPase Domain I

DETAILED DESCRIPTION OF THE INVENTION

A high throughput screening scintillation proximity assay (SPA - Amersham) was developed using PTP1B and a synthetic, biotinylated, ^{33}P-phosphorylated peptide as substrate. This peptide substrate, which corresponds to the activation loop of the insulin receptor kinase, i.e. Thr-Arg-Asp-Ile-Tyr-Glu-Thr-Asp-Tyr-Tyr-Arg-Lys-NH_2, was ^{33}P-phosphorylated on tyrosine residues using the insulin receptor tyrosine kinase. A compound library was screened and a number of hits identified. Surprisingly, one of these hits, oxalyl-amino benzoic acid proved to act as a classical, competitive, reversible active site-directed inhibitor (see below). 2-(Oxalyl-amino)benzoic acid was first described by (Friedlaender et al. Chem. Ber., 14, 1921 (1881)). However, despite that this compound has been known for decades there are no reports indicating any PTPase inhibitory activity.

First we analyzed the mode of inhibition of 2-(oxalyl-amino)benzoic acid and analogues thereof using classical steady-state enzyme kinetic methodology as described in R. A. Copeland, Enzymes - A Practical Introduction to Structure, Mechanism and Data Analysis, VCH Publishers, Inc., New York, 1996 (Figure 1). The illustrations are not intended in any way to limit the scope of the invention. In particular, it is not the intention to limit the scope of the invention to inhibitors that do not show any time dependence. Likewise, it is not the intention to limit the scope of the invention to classical, competitive inhibitors.

It appears from Figure 1 that some of the compounds of the invention (exemplified by oxalylamino benzoic acid) behave as a reversible, classical competitive inhibitor of PTP1B (linear relationship between inhibitor concentration and the apparent K_m (Fig. 1 (B); no influence on V_{max} (Fig. 1 (C)). The K_i value was found to be about 30 μM. The calculation of K_i is described in detail below and further illustrated by an example in the section DEFINITIONS.

We also investigated the influence of assay constituents, which have previously been found to significantly influence the inhibition of other PTPase inhibitors - as described above. EDTA was added to the assay buffer, and dithiothreitol was replaced by glutathione (Figure 2).

It appears from Figure 2 that the inhibitors of the present invention as an important feature, and in sharp contrast to the inhibitors described above, are insensitive to assay constituents such EDTA and the reducing agent (linear relationship between inhibitor concentration and the apparent K_m (Fig. 2 (B); no influence on V_{max} (Fig. 2 (C)). The K_i value was found to be about 50 μM. The reversible nature of the inhibition process is clearly indicated by the fact that V_{max} is independent of inhibitor concentration.

Further, it is demonstrated in Figure 3 that some of the compounds of the present invention do not show any sign of time dependency. Again, this shows the reversible nature of the inhibition.

We set out to identify more precisely the chemical elements defining the PTPase inhibitory capacity by analyzing a set novel chemical analogues. Importantly, as will be illustrated below, analogues of this hit (i.e. 2-(oxalyl-amino) benzoic acid) essentially retained the same enzyme kinetic profile, i.e. the behave like classical competitive inhibitors. Thus, the compounds of the invention can be derived by changing in a systematic fashion the elements needed for binding to/inhibition of/modulation of the active sites of PTPases and/or to other molecules with pTyr recognition units using procedures well known to those skilled in the art.

Examples of the enzyme kinetic behavior of analogues of 2-(oxalyl-amino)benzoic acid are shown in Figure 4 and 5. Surprisingly, these novel compounds retain the classical competitive mode of inhibition as observed with 2-(oxalyl-amino)benzoic acid. From this it appears that those skilled in the art may make novel analogues of the original compound that still act as inhibitors of protein-tyrosine phosphatases. As an example, which is not intended in any way to limit the scope of the invention, those skilled in the art may add substituents to 2-(oxalyl-amino)benzoic acid and

thereby change the potency and selectivity into other preferred compounds of the invention. Such novel compounds may be inhibitors or modulators of protein-tyrosine phosphatases or other molecules with pTyr recognition units and they may be classical, competitive inhibitors or mixed-type inhibitors. Thus, the present invention provides methods for making both non-selective and selective inhibitors and modulators of molecules with pTyr recognition units including protein-tyrosine phosphatases.

The compounds of the invention can be further modified to act as prodrugs.

It is a well known problem in drug discovery that compounds, such as enzyme inhibitors, may be very potent and selective in biochemical assays, yet be inactive in vivo. This lack of so-called bioavailability may be ascribed to a number of different factors such as lack of or poor absorption in the gut, first pass metabolism in the liver, poor uptake in cells. Although the factors determining bioavailability are not completely understood, there are many examples in the scientific literature - well known to those skilled in the art - of how to modify compounds, which are potent and selective in biochemical assays but show low or no activity in vivo, into drugs that are biologically active. It is within the scope of the invention to modify the compounds of the invention, termed the 'original compound', by attaching chemical groups that will improve the bioavailability of said compounds in such a way that the uptake in cells or mammals is facilitated. Examples of said modifications, which are not intended in any way to limit the scope of the invention, include changing of one or more carboxy groups to esters (for instance methyl esters, ethyl esters, acetoxymethyl esters or other acyloxymethyl esters). Compounds of the invention, original compounds, such modified by attaching chemical groups are termed 'modified compounds'. Said chemical groups may or may not be apparent in the claims of this invention. Other examples of modified compounds, which are not intended in any way to limit the scope of the invention, are compounds that have been cyclized at specific positions - socalled 'cyclic compounds' - which upon uptake in cells or mammals become hydrolyzed at the same specific position(s) in the molecule to yield the compounds of the invention, the original compounds, which are then said to be 'non-cyclic'. For the avoidance of doubt, it is understood that the latter original compounds in most cases will contain other cyclic or heterocyclic structures that will not be hydrolyzed after uptake in cells or mammals. Generally, said modified compounds will not show a be-

havior in biochemical assays similar to that of the original compound, i.e. the corresponding compounds of the invention without the attached chemical groups or said modifications. Said modified compounds may even be inactive in biochemical assays. However, after uptake in cells or mammals these attached chemical groups of the modified compounds may in turn be removed spontaneously or by endogenous enzymes or enzyme systems to yield compounds of the invention, original compounds. 'Uptake' is defined as any process that will lead to a substantial concentration of the compound inside cells or in mammals. After uptake in cells or mammals and after removal of said attached chemical group or hydrolysis of said cyclic compound, the compounds may have the same structure as the original compounds and thereby regain their activity and hence become active in cells and/or in vivo after uptake. A number of procedures, well known to those skilled in the art, may be used to verify that the attached chemical groups have been removed or that the cyclic compound has been hydrolyzed after uptake in cells or mammals. An example, which is not intended in any way to limit the scope of the invention, is given in the following. A mammalian cell line, which can be obtained from the American Tissue Type Collection or other similar governmental or commercial sources, is incubated with said modified compound. After incubation at conditions well known to those skilled in the art, the cells are washed appropriately, lysed and the lysate is isolated. Appropriate controls, well known to those skilled in the art, must be included. A number of different procedures, well known to those skilled in the art, may in turn be used to extract and purify said compound from said lysate. Said compound may or may not retain the attached chemical group or said cyclic compound may or may not have been hydrolyzed. Similarly, a number of different procedures - well known to those skilled in the art - may be used to structurally and chemically characterize said purified compound. Since said purified compound has been isolated from said cell lysate and hence has been taken up by said cell line, a comparison of said structurally and chemically characterized compound with that of the original unmodified compound (i.e. without said attached chemical group or said non-cyclic compound) will immediately provide those skilled in the art information on whether the attached chemical group as been removed in the cell or if the cyclic compound has been hydrolyzed. As a further analysis, said purified compound may be subjected to enzyme kinetic analysis as described in detail in the present invention. If the kinetic profile is similar to that of the original compound without said attached chemical group, but different

from said modified compound, this confirms that said chemical group has been removed or said cyclic compounds has been hydrolyzed.Similar techniques may be used to analyze compounds of the invention in whole animals and mammals.

A preferred prodrug is acetoxymethyl esters of the compounds of the present invention which may be prepared by the following general procedure *(C.Schultz et al, The Journal of Biological Chemistry,* **1993**, *268,* 6316-6322.*)*:

A carboxylic acid (1 equivalent) is suspended in dry acetonitrile (2 ml per 0.1 mmol). Diisopropyl amine (3.0 equivalents) is added followed by bromomethyl acetate (1.5 equivalents). The mixture is stirred under nitrogen overnight at room temperature. Acetonitrile is removed under reduced pressure to yield an oil which is diluted in ethylacetate and washed with water (3 x). The organic layer is dried over anhydrous magnesium sulfate. Filtration followed by solvent removal under reduced pressure afford a crude oil. The product is purified by column chromatography on silica gel, using an appropriate solvent system.

DEFINITIONS

Signal transduction is a collective term used to define all cellular processes that follow the activation of a given cell or tissue. Examples of signal transduction, which are not intended to be in any way limiting to the scope of the invention claimed, are cellular events that are induced by polypeptide hormones and growth factors (e.g. insulin, insulin-like growth factors I and II, growth hormone, epidermal growth factor, platelet-derived growth factor), cytokines (e.g. inter-leukins), extracellular matrix components, and cell-cell interactions.

Phosphotyrosine recognition units/tyrosine phosphate recognition units/pTyr recognition units are defined as areas or domains of proteins or glycoproteins that have affinity for molecules containing phosphorylated tyrosine residues (pTyr). Examples of pTyr recognition units, which are not intended to be in any way limiting to the scope of the invention claimed, are: PTPases, SH2 domains and PTB domains. Further, in some receptor-type or receptor-like PTPases, the second domain (the C-terminal domain) most likely does not pos-

sess catalytic activity. As a non-limiting example, the second domain of CD45 does not seem to act as an active PTPase (see Kashio et al., J. Biol. Chem. 273, 33856-22863 (1998) and references herein). However, the second domain of CD45 seems to play an important role as a phosphotyrosine recognition unit and to be critical for interleukin-2 secretion and substrate recruitment of TCRz in vivo (Kashio et al., supra). Thus, the second domain of CD45 in this case may play a similar role as a SH2 domain and hence act as a phosphotyrosine recognition unit. Although not formally proven, other molecules that are similar to PTPases, such as IA-2 and IA-2b, may act as pTyr recognition units.

Proteins with phosphotyrosine recognition units are defined as proteins or glycoproteins that contain phosphotyrosine recognition units.

A ligand is defined as a molecule or compound that binds to another molecule. An example of a ligand, which is not intended in any way to limit the scope of the definition, is a non-peptide molecule with a molecular weight equal to or below 2500 daltons which binds to a protein or a glycoprotein.

A phosphotyrosine recognition unit ligand is defined as a molecule that binds to the phosphotyrosine recognition unit(s) of a protein or a glycoprotein with phosphotyrosine recognition unit(s). Thus, non-limiting examples of a phosphotyrosine recognition unit ligand include PTPase inhibitors and/or PTPase modulators. Another non-limiting example of a phosphotyrosine recognition unit ligand is a compound that binds to an SH2 domain and/or to a PTB domain.

PTPases are defined as enzymes with the capacity to dephosphorylate pTyr-containing proteins or glycoproteins. Examples of PTPases, which are not intended to be in any way limiting to the scope of the invention claimed, are: 'classical' PTPases (intracellular PTPases (e.g. PTP1B, TC-PTP, PTP1C, PTP1D, PTPD1, PTPD2) and receptor-type PTPases (e.g. PTPα, PTPε, PTPβ, PTPγ, CD45, PTPκ, PTPμ), dual specificty phosphatases (VH1, VHR, cdc25), LMW-PTPases or acid phosphatases. A list of currently known classical and other PTPases reported to GenBank is shown in Table 1 (with the appropriate accession number indicated).

Table 1

List of PTPases (partial and full length cDNA)

Name	Accession Number				
Bovine					
bPTPBA14	U20807				
Chicken					
cLAR	L32780				
cPTP1B	U86410				
cPTPalpha	Z32749,L22437				
cPTPcryp2	U65891				
cPTPgamma	U38349				
cPTPlambda	L13285,Z21960				
cPTPsyp	U38620				
cPTPzeta	L27625				
Human					
hCD45	Y00638	Y00062	p08575		
hchPTP1	U42361				
hGLEPP1	U20489				
HLAR	Y00815				
hLCPTP	D11327				
hLyPTP1	AF001846				
hLyPTP2	AF001847				
hMEG1	M68941				
hMEG2	M83738				
hPCPTP1	D64053	U42361			
hPEST	D13380	M93425			
hPTP1B	M31724	M33689			
hSHP1	M74903	X62055	M77273	X82817* X82818**	
hSHP2	X70766	L08807	D13540	L03535	L07527
hPTP1E	U12128	D21209	D21210	D21211	
hPTPalpha	M34668	X54130	X54890	X53364	
hPTPBDP1	X79568				
hPTPbeta	X54131				
hPTPchlg	U77917	U77916*			
hPTPCOM1	Z79693				
hPTPD1	X79510				
hPTPD2	X82676				
hPTPdelta	X54133	L38929			
hPTPDEP1	U10886	D37781			
hPTPEC	X82635	E09724*			
hPTPepsilon	X54134				

Table 1 (continued)

List of PTPases (partial and full length cDNA)

Name	Accession Number				
hPTPFMI	X95712				
hPTPgamma	L09247	X54132			
hPTPH1	M64572				
hPTPHE	M64322				
hPTPIA2	L18983	Z48226			
hPTPIA2beta	U65065				
hPTPIAR	AF007555	L76258			
hPTPICA512	X62899				
hPTPkappa	L77886	Z70660			
hPTPL1	X80289				
hPTPmu	X58288				
hPTPPCP2	X97198				
hPTPp1	U81561				
hPTPPNP1	X79676				
hPTPps1	U60289	U73727			
hPTPrho	AF043644	AL024473	Al022239		AQ02047
hPTPRO	U71075				
hPTPS31	I32035	I32036	I32037	I32038	I32039
hPTPSAP1	D15049				
hPTPsigma	U35234	U40317	U41725		
hPTPU2	Z48541				
hPTPzeta	M93426	X54135	U88967		
hTCPTP	M25393	M81478	M80737		
hPTP-af007118	AF007118				
hchPTP	U42361				
Mouse					
mCD45	M14342	M92933	M33482		
mDPZPTP	D28529				
mLAR	Z37988				
mMEG2	Af013490				
mPEP	M90388				
mPEST	X86781				
mPTP1B	M97590	U24700			
mPTP1C	M68902	M90389			
mPTP1D	L08663	D84372			
mPTP35	X74438				
mPTP36	D31842				
mPTPalpha	M33671	M36033			

Table 1 (continued)

List of PTPases (partial and full length cDNA)

Name	Accession Number				
mPTPbeta	X58289				
mPTPBL	Z32740				
mPTPBR7	D31898				
mPTPbyp	D45212				
mPTPdelta	D13903	E09890	E09891	E09892	
mPTPepsilon	U35368	U36758	D83484	U62387	U40280
mPTPesp	U36488				
mPTPFLP1	U52523	U49853			
mPTPftp1	D88187				
mPTPgamma	L09562				
mPTPGMC1	AF073998	AF073999			
mPTPGMC1	AF073998	AF073999			
mPTPHA2	L40595				
mPTPIA2	U11812				
mPTPK1	U35124				
mPTPkappa	L10106				
mPTPlambda	U55057				
mPTPmu	X58287				
mPTPNP	U57345				
mPTPP19	X63440	S36169			
mPTPphi	U37467	U37466	U37465		
mPTPRIP	D83966				
mPTPRL10	D37801	D83072			
mPTPNU3	X82288				
mPTPT9	D28530	D28531*			
mPTPSL	Z30313	Z23058			
mPTPtestis	D64141				
mSTEP61	U28217	S80329	U28216		
mTCPTP	S52655	M81477	M80739		
Rat					
rCBPTP	M10072	Y00065			
rLAR	L11586	U00477	X83546	X83505	
rLCPTP	U28356				
rPC12PTP1	U14914				
rPTP- E10496	E10496				
rPTP-E09723	E09723				
rPTP1B	M33962				
rPTP1D	U09307	U05963			
rPTP20	U69673				

Table 1 (continued)

List of PTPases (partial and full length cDNA)

Name	Accession Number			
rPTP2E	U17971	U18293		
rPTPalpha	L01702			
rPTPBEM1 (partial cds)		D45412		
rPTPBEM2	D45413			
rPTPBEM3	D45414			
rPTPD30 (complet cds)		U28938		
rPTPDEP1	U40790			
rPTPepsilon	D78610	D78613		
rPTPGMC1	AF063249			
rPTPICA105	X92563	D38222		
rPTPNE6	U73458	Z50735		
rPTPOST	L36884			
rPTPP1	L19180			
rPTPPS	L19181			
rPTPpsi	U66566			
rPTPsigma	L11587	L12329	L19933	
rPTPzeta	U09357			
rRKPTP	D38072			
rSTEP	S49400			
rSHP1	U77038			
rTCPTP	X58828			
rPTPTD14	AF077000			
Rabbit				
rabPTP-oc	U32587			
Other PTPases				
VH1-like (ie both dual + tyrosine)				
hCL100	X68277	S46269		
hMKP-2	U48807			
hMKP-4	Y08302			
hPAC-1	U23853	L11329		
hPTEN	U93051	AF000731	AF000732	AF000733
	AF000734			
hPTPpyst1	X93920			
hPTPpyst2	X93921			
hPTPTH2	AF019083			

Table 1 (continued)

List of PTPases (partial and full length cDNA)

Name	Accession Number	
hTYP1	S80632	
hVH1	???	
hVH2	U21108	
hVH5	U27193	
hVHR	???	
mERP	S64851	
mI29-PAC1	U09268	
mMKP-1	X61940	
mNTTP1	X95518	
mPAC-1	L11330	
mPRL-1	U84411	
mSty	U11054	
mSTYX	U34973	
mVH1	X61940	
myxomaPTP	L31960	
nostocPTP	L11392	
raccPTP	L13165	
rCL100	S81478	X84004
rMKP-2	U23438	
RPRL-1	L27843	
ru02553	U02553	
rVH6	U42627	
shopePTP	L32180	
yVH1	L04673	
hPRL3	AF041434	
LMW		
bPTP1mw	M83656	
hRBC1mw	M83653	M83654
yLMPTP1	L33929	
yLTP1	U11057	L48604
cdc25-family:		
hPTPcdc25a	M81933	
hPTPcdc25b2	Z68092	
hcdc14B	AF023158	

Table 1 (continued)

List of PTPases (partial and full length cDNA)

Name	Accession Number
Unclassified mammalian PTPases:	
hPTPCAAX1	U48296
hPTPCAAX2	U48297
hPTPCIP2	L25876
hPTPCdi1	U02681
hPTPICAAR	Y08569
hPTPTEP1	U96180
hPTPkiaa0283	AB006621
hPTPPRL-1	AF051160
hPTPPRL-3	AF041434
hPTP_putative_	AF007118
mPTP-IF1	Y17345
mPTP-IF2	Y17344
mPTP-IF2P	Y17343
Microbial PTPases (ie. eubacteria & vira)	
autovPTP	M96763
salmPTP	U63293
strepPTP	U37580
Rachiplusia ou nuclear polyhedrosis virus	
npvPTP	AF068270
Oher eukaryotes (ie. Drosophila, yeast, fungi, xenopus etc.)	
Arabidopsis thaliana	
atPTP1	AF055635
atPTP1-exons-introns	AJ006309
Caenorhabditis elegans	
ceCosmid	Z80216
cePTP2	AF015882
cePTP6	Z70284
cePTPclr-1	AF047880
cePTPPRL-1	AF063401

Table 1 (continued)

List of PTPases (partial and full length cDNA)

Name	Accession Number			
Drosophila				
dLAR	M27700			
dPTP10D	M80465	M80538		
dPTP4E	L20894			
dPTP61F	L11253	L14849	L11252	L11251
dPTP69D	M27699			
dPTP99A	M80464	M80539	M81795	
dPTPcork	U19909			
dPTPPRL-1	AF047880	AF047881		
Dictyostelium discoideum				
dictPTP	L07125			
dictPTP2	L15420			
dictPTP3	U38197			
Emericella nidulans				
fPTPncdc25	X64601	S37934		
Tritrichomonas foetus				
triPTP.pep	U66070			
Schizosaccharomyces pombe				
yPTP1	Z73100			
yPTPcand	L01038			
yPTPMSG5	D17548			
yPTPpyp1	M63257			
yPTPpyp2	X59599	S51320		
yPTPpyp3	X69994	S51385		
yPTPtrna	X75077			

Table 1 (continued)

List of PTPases (partial and full length cDNA)

Name	Accession Number
Yersinea cerivisiae	
yscPTP	M64062
yscPTP2	M38723 M82872
yscPTP3	AF006304
Xenopus	
xCD45	AF024438
xPTP-SH2	U15287
xPTPalpha	U09135
xPTPX1	L33098
xPTPX10	L33099
Hirudo medicinaliS	
hmLar1	AF017084
hmLar2	AF017083
Pisum sativum (pea)	
peaPTP1	AJ005589
Glycine max (soybean)	
soybeanPTP1	AJ006308

A modulator of PTPases is compound that causes a change of the activity of a PTPase. PTPase modulators may either make the PTPase less active or more active. PTPase modulators may according to the present definition bind to the active site of PTPases or to areas outside the active site of PTPases (so-called allosteric modulators). Another, non-limiting example of a PTPase modulator is a compound that changes the substrate specificity of a PTPase.

A **PTPase domain** is defined as a part of the full PTPase molecule, which typically - but not always - possess characteristic enzymatic activity, i.e. the capacity to dephosphorylate pTyr-containing proteins or glycoproteins. A PTPase domain of a classical PTPase will typically consist of 220-350 amino acid residues and correspond to amino acid residues number 30 to 270 of PTP1B. PTPase domains may be expressed in eukaryotic and prokaryotic expression system either as the domain itself or as part of a fusion protein.

SH2 domains Src homology 2 (SH2) domains are non-catalytic protein modules that bind to pTyr (phosphotyrosine residue) containing proteins, i.e. SH2 domains are pTyr recognition units. SH2 domains, which consist of ~100 amino acid residues, are found in a number of different molecules involved in signal transduction processes. The following is a non-limiting list of proteins containing SH2 domains: Src, Hck, Lck, Syk, Zap70, SHP-1, SHP-2, STATs, Grb-2, Shc, p85/PI3K, Gap, vav (see Russell et al, FEBS Lett. 304:15-20 (1992); Pawson, Nature 373: 573-580 (1995); Sawyer, Biopolymers (Peptide Science) 47: 243-261 (1998); and references herein).
The structural requirements for SH2 domain/pTyr protein interactions have been analyzed with synthetic, tyrosine phosphorylated peptides and further elucidated by X-ray crystallography and NMR. The signature motif, FLVRES (single amino acid code), forms part of the binding pocket for pTyr. In addition, other binding pocket(s) play(s) a role in defining affinity and selectivity. Thus, in the Src SH2 domain a hydrophobic pocket binds the amino acid positioned 3 residues C-terminal to the pTyr residue (i.e. pY+3). Numerous studies have pointed to the importance of residues positioned C-terminal to the pTyr residue (for review, see Pawson, *supra*).
Determination of ligand binding to SH2 domains. Several methods – well known to those skilled in the art - have been developed that are useful for assessment of the binding of non-phosphate-containing ligands to SH2 domains. One example, which is

not intended in any way to limit the scope of the present invention, was recently published by Yao and coworkers (Yao et al., J. Med. Chem. 42: 25-35 (1999)). These authors used the surface plasmon reasonance method for determination of the inhibitory capacity of non-phosphate containing ligands on Grb2 SH2 domain binding (IC50). In brief, recombinant GST-Grb2 SH2 domain was incubated with various amounts of ligand and allowed to flow across the surface-bound the SHC phosphopeptide, DDPSpYVNVQ (single amino acid code, where pY indicates phosphotyrosine). The amount of equilibrium binding (Ru(max)) was determined and compared to binding without the presence of ligand. The above SHC phosphopeptide, DDPSpYVNVQ, served as a control. Similarly, ligand binding can be determined for other SH2 domains using appropriate surface-bound tyrosine-phosphorylated peptides well known to those skilled in the art.

Other non-limiting approaches to assess peptide ligand binding to SH2 domains have been developed by several laboratories (Fantl et al., Cell 69: 413-423 (1992); Ward et al., J. Biol. Chem. 271: 5603-5609 (1996); and references herein). Such assays can also be used for assessment of the binding of non-peptide ligands to SH2 domains with slight modifications well understood by those skilled in the art.

PTB domains Recently, a novel type of pTyr recognition unit (PTP domain = phosphotyrosine binding domain) was identified in shc, which is an adaptor protein (Kavanaugh and Williams, Science 266: 1862-1865). The PTB domains are longer than SH2 domain (~ 190 residues). Assays for ligand binding to the shc PTB domain was recently developed by Kavanaugh and coworkers (Kavanaugh et al., Science 268: 1177-1179 (1995); Laminet et al., J. Biol. Chem. 271: 264-269 (1996)).

A **PTPase family** is defined as a group of PTPases that are structurally related. Thus, one accepted way of defining a PTPase family is based on the primary structures of PTPases outside the PTPase domain(s) or their overall structures (Fischer et al. (1991) *Science 253:* 401-406; B.J. Goldstein (1995) in Protein Profile, volume 2, number 13, Academic Press Ltd., London.. Non-limiting examples of PTPase families defined in such way are:

(a) SH-2 domain containing PTPases, the SHP family: SHP-1; SHP-2

(b) PTP1B family: PTP1B; TC-PTP

(c) Ezrin-domain containing PTP family: PTPH1; PTPD1; PTPD2; PTPMEG

(d) The PTP-BAS family

(e) Proline-Glutamic acid-Serine-Threonine (PEST) sequence containing PTPases: PTP-PEST; PEP

(f) PTPases containing very small, highly glycosylated extracellular regions, the PTPα family: PTPα; PTPε.

(g) Receptor-type PTPases with one intracellular PTP domain, the PTPβ family: PTPβ, DEP-1, GLEPP-1, SAP-1

(h) PTPases containing extracellular regions with immunoglobulin-like domains and Fibronectin III-like domains, the PTP-LAR family: PTP-LAR; PTPσ; PTPδ.

(i) PTPases containing extracellular regions with MAM domains, the PTPμ family: PTPμ; PTPκ.

(j) PTPases containing extracellular regions with similarity to carbonic anhydrase, the PTPζ family: PTPζ, PTPγ

(k) The IA-2 family

(l) The PTPψ family

(m) The CD45 family

It should be noted that not all PTPases have been - or can be - classified into any families.

As an alternative, PTPases may also be divided into families based on sequence alignments of the primary sequence (Goldstein *vide supra*). Computer programs well-known to those skilled in the arts (e.g. GCG University of Wisconsin, refs.) may be used to performed such alignments. Further analysis is performed with computer programs such as CLUSTALX resulting in a so-called phylogenetic tree. An example of said phylogenetic tree is shown in Figure 7. It should be pointed out that the above described ways of dividing PTPases into families show considerable overlap. The preferred definition of PTPases into families is the latter based on primary sequence alignments of PTPases, since it is likely that this definition in turn can be used to establish assays that will allow development of PTPase inhibitors or modulators that selectively react with a given PTPase family or members of a specific family (i.e. selective inhibitors).

Modulation of cellular processes is defined as the capacity of compounds of the invention to 1) either increase or decrease ongoing, normal or abnormal, signal transduction, 2) initiate normal signal transduction, and 3) initiate abnormal signal transduction.

Modulation of pTyr-mediated signal transduction/modulation of the activity of molecules with pTyr recognition units is defined as the capacity of compounds of the invention to 1) increase or decrease the activity of proteins or glycoproteins with pTyr recognition units (e.g. PTPases, SH2 domains or PTB domains) or to 2) decrease or increase the association of a pTyr-containing molecule with a protein or glyco-protein with pTyr recognition units either via a direct action on the pTyr recognition site or via an indirect mechanism. Examples of modulation of pTyr-mediated signal transduction/modulation of the activity of molecules with pTyr recognition units, which are not intended to be in any way limiting to the scope of the invention claimed, are: a) inhibition of PTPase activity leading to either increased or decreased signal transduction of ongoing cellular processes; b) inhibition of PTPase activity leading to initiation of normal or abnormal cellular activity; c) stimulation of PTPase activity leading to either increased or decreased signal transduction of ongoing cellular processes; d) stimulation of PTPase activity leading to initiation of normal or abnormal cellular activity; e) inhibition of binding of SH2 domains or PTB domains to proteins or glycoproteins with pTyr leading to increase or decrease of ongoing cellular processes; f) inhibition of binding of SH2 domains or PTB domains to proteins or glycoproteins with pTyr leading to initiation of normal or abnormal cellular activity.

A subject is defined as any mammalian species, including humans.

DEFINITION OF PTPase INHIBITION ACCORDING TO THE PRESENT INVENTION

A compound is defined as a PTPase inhibitor if the following criteria are fulfilled: (a) the inhibitory capacity must be determined as described in detail below and the inhibition constant, K_i, value must be below 1000 μM; (b) at least one PTPase must be inhibited by the compounds of the invention. Any PTPase may be used for the analyses. Non-limiting examples of PTPases are: PTP1B; SHP-1, SHP-2; PTP-PEST;

PTPα; PTPμ; LAR; CD45. Further examples are given in Table 1. In addition, any PTPase not mentioned herein may be used.

DETERMINATION OF INHIBITOR CONSTANTS

Determination of inhibitor constants (K_i values) may be performed according to a number of different experimental procedures including inhibitor fluorescence quenching. However, in all cases, to evaluate the compounds of the present invention such methods must be supplemented with procedures that measure the effect of the compounds on the catalytic activity of the enzymes. The conditions for such assays are illustrated below.

PTPases

PTPases used for the analyses may be expressed as intact molecules or as PTPase domains.

Assay conditions

Assay conditions must be selected to ensure enzyme stability, i.e. the enzyme must in the absence of substrate retain at least 50 per cent of the initial activity over the assay period.

Buffer systems

Any buffer system well known to those skilled in the art may be selected for analysis of compounds of the invention. Preferred buffers used for analysis of PTPase inhibition or modulation are listed below.

Buffer 1

100 mM NaAc (sodium acetate) pH 5.5

0.1 % BSA (bovine serum albumin)

15 mM DTT (dithiothreitol)

Buffer 2

100 mM NaAc pH 5.5

50 mM NaCl
0.1 % BSA
5 mM DTT

Buffer 3
100 mM NaAc pH 5.5
50 mM NaCl
0.1 % BSA
5 mM GSH (glutathione)
1 mM EDTA

Buffer 4
50 mM HEPES pH 7.0
100 mM NaCl
0.1 % BSA
5 mM DTT

Buffer 5
50 mM HEPES pH 7.0
100 mM NaCl
0.1 % BSA
5 mM GSH
1 mM EDTA

Buffer 6
20 mM MES pH 6.0
150 mM NaCl
5 mM DTT
0.1 % BSA

Buffer 7
(constant ionic strength buffer described by Ellis & Morrison (1982) *Methods Enzymol. 117:* 301-342)
50 mM Tris

50 mM Bis-Tris

100 mM acetate

pH range: 4.5 - 9.0

with or without reducing agents (DTT, GSH, 2-mercaptoethanol)

with or without carrier proteins (e.g. BSA, gelatine)

Reaction time

The reaction time is preferred to be between 2 and 60 minutes.

Reaction temperature

Any reaction temperature well known to those skilled in the art may be selected for analysis of compounds of the invention. The preferred temperature is in the following range: 4° C to 37° C.

Substrates

Substrates used in the reaction may be selected from the following: (a) p-nitrophenyl phosphate (pNPP); (b) tyrosine-phosphorylated peptides; (c) natural substrates (e.g. autophosphorylated insulin receptor) or parts thereof (e.g. autophosphorylated tyrosine kinase domain of the insulin receptor). When pNPP is used as substrate the enzyme reaction is followed by measurement of the optical density at a wavelength of approximately 405 nm. In case of (b) and (c), the enzyme reaction may be followed by measurement of released phosphate or by spectrophotometric/fluorometric methods according to procedures well known to those skilled in the art.

Concentration of compound and substrate

To ensure optimal determination of the inhibitor constants the concentration of substrate and inhibitor must be varied independently according to the following guidelines.

A range of substrate concentrations must be varied with a preferred maximum, final assay concentration at least 10-fold above that of the K_m value for the enzyme determined under the same conditions. The minimum final assay concentration is pref-

erably equal to or below that of the K_m value for the enzyme determined under the same conditions.

At least 2 different inhibitor concentrations must be used. The concentrations will depend on the actual compounds, but they must be selected in such a way that non-linear regression analysis allows determination of inhibitor constants with an accuracy acceptable to those skilled in the art.

Calculation of inhibitor constants, K_i

Definitions

V_o, the initial velocity, is the reaction corresponding to time zero.

K_m is defined as the concentration of substrate used to obtain an initial velocity corresponding to 50 percent of the maximal obtainable velocity (V_{max}) at full substrate saturation of the enzyme. K_m is measured without addition of inhibitor.

V_{max} is the maximum obtainable initial velocity (limiting rate) determined at full substrate saturation.

K_{app} is the apparent K_m value determined in the presence of inhibitor.

V_{app} is the apparent V_{max} value determined in the presence of inhibitor.

Competitive inhibitors are defined as compounds that bind to the substrate binding site of the enzyme, or in close enough proximity to occlude the substrate binding site. True competitive inhibitors, also termed classical competitive inhibitors, increase **K_{app}** without any effect on **V_{max}**.

Mixed-type inhibitors are defined as inhibitors that affect both **K_m** and **V_{max}**.

Non-competitive inhibitors are defined as inhibitors that decrease V_{app} without any effect on K_m.

In accordance with classical Michaelis-Menten kinetics, the inhibitor constant of competitive inhibitors, K_i, may be calculated from the following equation

$$K_{app} = (K_m / K_i)*[i] + K_m \quad \text{(equation 1)}$$

where **[i]** is the concentration of the inhibitor

The competitive part of K_i of mixed-type inhibitors, K_ic, may be calculated from

$$K_ic=i/((V_m/K_m)*(K_{app}/V_{app})-1) \quad \text{(equation 2)}$$

The uncompetitive part of K_i of mixed-type inhibitors, K_iu, may be calculated from

$$K_iu=i/(V_{max}/V_{app}-1) \quad \text{(equation 3)}$$

The K_i values for a given compound may be calculated either using linear transformation procedures or nonlinear regression fit to classical Michaelis Menten enzyme kinetic models as defined above assuming competitive or mixed-type inhibition. Preferred compounds of the invention belongs to the class of competitive or mixed-type inhibitors.

Further information on the definitions of the above enzyme kinetic parameters may be found in any reference book on enzyme kinetics. Non-limiting examples of such reference books are: (a) Copeland (*vide supra*); (b) M. Dixon & E.C. Webb, Enzymes, 2nd Edition, Longmans, London, 1996; (c) A. Cornish-Bowden, Fundamentals of Enzyme Kinetics, Portland Press, 1995.

Calculation of inhibitor constants - an example

The calculation of K_i values may become more apparent by the following example, which is not intended in any way to limit the scope of the invention.

PTP1B was incubated with a compound of the invention (described in Example 2).

A truncated form of PTP1B, corresponding to the N-terminal 321 amino acids was expressed in E. coli and purified to apparent homogeneity using published procedures well-known to those skilled in the art. The enzyme reactions were carried out using standard conditions essentially as described by Burke *et al* (*Biochemistry 35; 15989-15996 (1996)*). The assay conditions were as follows.

Half of a 96-well plate was used for this experiment. *p*-nitrophenyl phosphate (pNPP) was used as substrate (see Table 2). The following final assay concentrations of pNPP were used: 10 mM (added to all wells in row A), 5 mM (added to all wels in row B), 2.5 mM (added to all wells in row C), 1.25 mM (added to all wells in row D), 0.63 mM (added to all wells in row E), 0.31 mM (added to all wells in row F), 0.16 mM (added to all wells in row G). No substrate was added to row H (a volume of assay buffer corresponding to that of pNPP was added to all wells in row H). 3-(Oxalylamino)naphthalene-2-carboxylic acid dissolved in DMSO (Example 2) was used as inhibitor and used at the following final assay concentrations: 100 μM (added to all wells in column 1), 33.3 μM (added to all wells in column 2); 11.1 μM (added to all wells in column 3); 3.7 μM (added to all wells in column 4). Assay buffer was added in columns 5 and 6 instead of the inhibitor (same volume as the inhibitor in columns 1-4). Assay buffer (final assay concentration): 100 mM sodium acetate pH 5.5, 50 mM NaCl, 5 mM glutathione, 1 mM EDTA, and 0.1 % bovine serum albumin. The reaction was started by addition of the enzyme, PTP1B. Assay buffer was added in column 6 instead of the enzyme (same volume as the enzyme in columns 1-5). The total volume in each well was 100 μl, including 10 μl of inhibitor dissolved in DMSO or 10 μl DMSO added to the control wells that did not receive inhibitor. The temperature was 25 °C. After 60 minutes, NaOH was added and the absorbance was read at 405 nm. The results are shown in Table 2.

Table 2

Absorbance measured at 405 nm

	1	2	3	4	5	6
A	1.226	1.369	1.445	1.461	1.469	0.124
B	0.929	1.172	1.33	1.35	1.429	0.084
C	0.623	0.971	1.17	1.25	1.311	0.064
D	0.426	0.718	0.948	1.047	1.139	0.053
E	0.254	0.45	0.651	0.755	0.837	0.048
F	0.142	0.266	0.394	0.454	0.501	0.044
G	0.095	0.159	0.226	0.259	0.287	0.044
H	0.043	0.041	0.044	0.042	0.043	0.044

Calculation of K_i values

The actual data from Table 2 are shown with the assay setup in Table 3.

Table 3

	Inhib					noEnz
pNPP	100.0	33.3	11.1	3.7	0.0	0.0
10.00	1.226	1.369	1.445	1.461	1.469	0.124
5.00	0.929	1.172	1.33	1.35	1.429	0.084
2.50	0.623	0.971	1.17	1.25	1.311	0.064
1.25	0.426	0.718	0.948	1.047	1.139	0.053
0.63	0.254	0.45	0.651	0.755	0.837	0.048
0.31	0.142	0.266	0.394	0.454	0.501	0.044
0.16	0.095	0.159	0.226	0.259	0.287	0.044
0	0.043	0.041	0.044	0.042	0.043	0.044

First the absorbance measurement have to be corrected for any OD_{405} value derived from the control well H6, as shown in Table 4.

Table 4

	Inhib					noEnz
pNPP	100.0	33.3	11.1	3.7	0.0	0.0
10.00	1.182	1.325	1.401	1.417	1.425	0.08
5.00	0.885	1.128	1.286	1.306	1.385	0.04
2.50	0.579	0.927	1.126	1.206	1.267	0.02
1.25	0.382	0.674	0.904	1.003	1.095	0.009
0.63	0.21	0.406	0.607	0.711	0.793	0.004
0.31	0.098	0.222	0.35	0.41	0.457	0
0.16	0.051	0.115	0.182	0.215	0.243	0
0	-0.001	-0.003	0	-0.002	-0.001	0

The values in wells H1 through H5 indicated any color (OD_{405} value) derived from the inhibitor itself. In the present example, the inhibitor does not give rise to any OD_{405} value. The values in column 6 indicate the OD_{405} values due to the absorbance of the substrate. Therefore, the corrected OD_{405} values in Table 4 must be further corrected for the absorbance at 405 nm which is caused by the inhibitor and/or the substrate, as shown in Table 5.

Table 5

	Inhib					noEnz
pNPP	100.0	33.3	11.1	3.7	0.0	0
10.00	1.103	1.248	1.321	1.339	1.346	0
5.00	0.846	1.091	1.246	1.268	1.346	0
2.50	0.56	0.91	1.106	1.188	1.248	0
1.25	0.374	0.668	0.895	0.996	1.087	0
0.63	0.207	0.405	0.603	0.709	0.79	0
0.31	0.099	0.225	0.35	0.412	0.458	0
0.16	0.052	0.118	0.182	0.217	0.244	0
0	0	0	0	0	0	0

The corrected values in Table 5 are now used for calculation of the K_i values of the inhibitor at each of the analyzed inhibitor concentrations (100, 33.3, 11.1 and 3.7 µM, respectively). Classical Michaelis-Menten plots of these data are shown in Figure 4 (A). Using non-linear regression analysis of the data in Table 5, the apparent K_m val-

ues (in mM; K_{app}) and apparent V_{max} values (OD_{405} values; V_{app}) are calculated for each inhibitor concentration (Table 6).

Table 6

Inh	100	33.3	11.1	3.70	0
Kapp	4.30	1.53	0.89	0.69	0.59
Vapp	1.57	1.44	1.47	1.46	1.50

These data are shown graphically in Figure 4 (B) and (C).

Using the data in Table 6 and Equation 1 (see above) the K_i values can be calculated for each inhibitor concentration (Table 7).

Table 7

Inhibitor concentration (μM)	100.0	33.3	11.1	3.7
Ki (μM)	16.0	21.1	22.5	23.0

It appears that the K_i values are almost the same, irrespective of the inhibitor concentration. When combining this with the fact that the inhibitor does not change the V_{max} value it can be concluded that this inhibitor is a classical, competitive inhibitor. This is further illustrated by calculating competitive part of K_i (K_ic) assuming mixed-type inhibition and using Equation 2 (see above). If the inhibitor is mixed-type then the calculated K_ic values should differ significantly from the K_i values in Table 7. The K_ic values for the present inhibitor is shown in Table 8.

Table 8

Inhibitor concentration (μM)	100.0	33.3	11.1	3.7
Kic	17.0	19.8	21.1	19.9

It appears that the K_ic values in Table 8 are almost identical to the K_i values in Table 7.

Selectivity of an inhibitor is defined as the property of such compound to inhibit or modulate a certain PTPase or certain PTPases more efficiently than other PTPases. A selective inhibitor may inhibit one PTPase only or one PTPase family only. However, other selective inhibitors also include compounds that inhibit a set of several PTPases or PTPase families more efficiently than other sets of PTPases or PTPase families.

An example of selectivity, which is not intended in any way to limit the scope of the invention, is a competitive inhibitor that has a K_i value of 50 μM against PTP1B and a K_i value of 500 μM or more against PTPα. An example of a selective modulator, which is not intended in any way to limit the scope of the invention, is a modulator that causes a 2-fold increase of the activity of SHP-1 without affecting the activity of PTPα.

To further illustrate the use of different PTPases in evaluating the potency of an inhibitor, a brief description will be given of how to make and express other PTP constructs. For those skilled in the art, this description will allow expression and purification of other PTPase domains that can be used for evaluation of potency as well as selectivity (vide infra) of PTPase inhibitors. In brief, appropriate sources of tissue or cells or cell lines are used to isolate RNA (total or messenger RNA). As non-limiting examples, RNA can be isolated from placenta, liver, skeletal muscle, fat tissue, and peripheral blood leukocytes. Using standard procedures (Ausubel, F. M., et al. (Eds.). SHORT PROTOCOLS IN MOLECULAR BIOLOGY, 2nd EDITION: A compendium of methods from Current Protocols in Molecular Biology. John Wiley and Sons, Inc. New York ISBN 0-471-57735-9 (1992); Ausubel, Frederick M. Current Protocols on CD-ROM User's Guide; Current protocols in molecular biology. John Wiley and Sons, Inc. (1998).), well known to those skilled in the art, cDNA was prepared from appropriate RNA preparations. Such cDNA preparations in turn served as template for the polymerase chain reaction (PCR). It should further be noted that appropriate cDNA templates can be obtained from commercial sources such as Clontech (1020 East Meadow Circle, Palo Alto, CA 94303). The PCR technique was used to prepare cDNA corresponding to the following PTPase domains (Ausubel et al., supra): PTP1B; PTPα domain 1; PTPε domain 1; PTPβ; CD45 domain 1 and 2. Ap-

propriate restriction sites have been included in the oligonucleotides to allow cloning into appropriate expression vectors. In these examples, which are not intended in any way to limit the scope of the invention, the pGEX expression vectors (Pharmacia) were used. For convenient cloning into other expression vectors (not shown here), an additional N-terminal methionine (Met – M) was included in some of the constructs (indicated as (M)). All sequences were confirmed by sequencing of both strands. Further details are given in Table 9. The information on the oligonucleotides used for the PCRs and the GenBank accession numbers for the specific PTPases in question will allow those skilled in the art to obtain cDNA encoding these PTPase domains. After insertion into the appropriate expression vectors, Escherichia coli was transformed with the expression vectors encoding the above glutathione-S-transferase (GST) fusion proteins. Overnight cultures were diluted 1:25 and grown for 3 hrs at 37 oC. Expression of GST fusion proteins was then induced by addition of isopropyl-1-thio-b-D-galactopyranoside, and the cultures were grown for additional 3 hrs at room temperature. The GST fusion proteins were purified according to the manufacturer's instructions (Pharmacia) with minor modifications. In brief, all purification steps were conducted at approx. 4 °C. The cell pellets were suspended (5 ml/g) in lysis buffer (50 mM imidazole, 5 mM EDTA, 0.1 % b-mercaptoetanol, 10 % glycerol, 10 µg/ml aprotinin, 0.1 % lysozyme and 1 mM PMSF; pH 7.2) by stirring for 1h prior to lysis under nitrogen pressure (>2000 psi) in a Parr cell disruption bomb. Triton X-100 (0.1 %) was added to the lysate and stirring continued for 1h prior to centrifugation at 40000 g for 30 min. The supernatant was applied to a Glutathione Sepharose column (Pharmacia) equilibrated with GST-equilibration buffer (50 mM imidazole, 1 mM EDTA, 150 mM NaCl and 10 % glycerol; pH 7.2) and initially washed with the same buffer. The flow direction was changed and washing was continued with a washing buffer (50 mM Tris, 1 mM EDTA and 10 % glycerol; pH 8). Finally, the bound protein was eluted with 10 mM glutathione in the washing buffer. The CD45 fusion protein was further purified on G25 and Mono Q columns (Pharmacia). The purified PTP domains are stored at –80 °C until use. Immediately prior to use the enzyme preparations are diluted appropriately.

It should be noted that similar methods - well known to those skilled in the art - could be used to obtain the catalytic domains of the molecules shown in Table I.

Said GST-PTPase fusion proteins are used to assess the potency and selectivity of PTPase inhibitor essentially as described for PTP1B above. To further illustrate

these assays, a non-limiting example using PTPα domain 1 will be given. Similar procedures may be used for other PTPase domains. Half of a 96-well plate was used for this experiment, p-nitrophenyl phosphate (pNPP) was used as substrate. The following final assay concentrations of pNPP were used: 20 mM, 10 mM, 5 mM, 2.5 mM, 1.25 mM, 0.63 mM, 0.31 mM. The compound, 5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid, dissolved in dimethylsulfoxide (DMSO) was used as inhibitor and used at the following final assay concentrations: 200 μM, 66.6 μM; 22.2 μM; 7.4 μM. Assay buffer instead of enzyme, and/or substrate was added to appropriate control wells as described in detail for PTP1B above. Assay buffer (final assay concentration): 100 mM sodium acetate pH 5.5, 50 mM NaCl, 5 mM glutathione, 1 mM EDTA, and 0.1 % bovine serum albumin. The reaction was started by addition of the enzyme, GST-PTPα domain 1 (final dilution 1:10000). The assay total volume in each well was 100 μl, including 10 μl of inhibitor dissolved in DMSO or 10 μl DMSO added to the control wells that did not receive inhibitor. The temperature was 25 °C. After 60 minutes, 10 μl of a 0.5 M sodium hydroxide solution (in 50 percent (vol/vol) ethanol) was added to each well and the absorbance was read at 405 nm. The results are shown in Figure 6A. The calculated K_i value is 4 μM (median value). Figure 6B shows the results when the same compound (5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid) is tested against PTPα (final dilution 1:2000) in the following buffer: 50 mM HEPES pH 7.0, 100 mM NaCl, 5 mM glutathione, 1 mM EDTA, and 0.1 % bovine serum albumin. After 60 minutes, 20 μl of a 0.5 M sodium hydroxide solution (in 50 percent (vol/vol) ethanol) was added to each well and the absorbance was read at 405 nm. Otherwise, the conditions are as described for Figure 6A. The calculated K_i value under these conditions is about 70 mM (median value).

Table 9

PTPase	Vector	Amino acids no.	Nucleotides no.	GenBank Acc.no.	Forward PCR primer	Reverse PCR primer
PTP1B	pGEX	1-321	92-1052	M31724	5' CG CCCTGGCCCCATATGGA-GATGGAAAAGGAGTTCGAG 3'	5' AACTCTAGAGGATCC-TAATTGTGTGGCTCCAGGATTCG 3'
PTPα domain 1	pGEX-4T-1	(M)R206-499	649-1531	X54130	5' CCCCCCGGGCATATGAG-GAAATACCCACCCCTGC 3'	CTAGAGATCTCACTTACAGTTCTGTATC
PTPε domain 1	pGEX-5X-2	(M)K 110-402	379-1257	X54234	5' TCACGGATCCGGATGAAGAAG-TATTTTCCCATCCCCGTGG 3'	5' AGGGAGAAGCTTCA-CAGCTCTGTGTCCCCGTAGAGCATC 3'
PTPβ	pGEX-5X-2	(M)Q 1644-1998	4961-6021	X54131	5' GTTGCCTTACATATGGGGATC-CAGAAAGTGAGCCATGGTCGAG 3'	5' CCGCTCGA-GATCTCAATGCCTTGAATAGACTGGATC 3'
CD45 domain 1+2	pGEX-5X-2	467-1145	1539-3575	Y00062	5' GAGAGATCTCTCATAT-GAATGTGGAGCCAATCCATGC 3'	5' CCGCTCGAGATCTAT-GAACCTTGATTTAAGGCTGG 3'

A **selective inhibitor** is defined as an inhibitor that shows selectivity.

A **non-selective inhibitor** is defined as an inhibitor that does not show selectivity.

A **selective modulator** is defined as a modulator that shows selectivity.

To further illustrate the concept of selective and non-selective PTPase inhibitors an example of a non-selective and a selective inhibitor, respectively, is provided in Table 10. It should emphasized that the examples in Table 10 are not intended in any way to limit the scope of the invention.

Table 10

Analysis of selectivity of PTPase inhibitors. Assay conditions are essentially the same as those used in Figure 4. The numbers given are K_i values (μM).

	Compound of Example 82	Compound of Example 83
PTP1B	10	2
PTP-LAR	1000	>1000
PTPε	50	1000
CD45	50	300
PTPβ	30	>1000

It appears from Table 10 that the compound of example 82 is an example of a non-selective inhibitor, whereas the compound of example 83 behaves like a selective inhibitor. It should be noted that the compound in example 83 when tested against other PTPases might be inhibitory against these. Yet, according to the present definition, the compound in example 83 is a selective inhibitor due to the fact that it inhibits PTP1B with little effect on the other PTPases tested in Table 10. Also, according to the present definition, the compound in example 82 is a non-selective inhibitor due to its inhibitory capacity against several PTPases, even though it has weak activity, if any, against PTP-LAR.

A **chemical group** is defined as any single atom or any group of covalently linked atoms or any molecule, including any radical thereof.

The terms "halogen" or "halo" include fluorine, chlorine, bromine, and iodine.

The term "alkyl" includes C_1-C_6 straight chain saturated and C_2-C_6 unsaturated aliphatic hydrocarbon groups, C_1-C_6 branched saturated and C_2-C_6 unsaturated aliphatic hydrocarbon groups, C_3-C_6 cyclic saturated and C_5-C_6 unsaturated aliphatic hydrocarbon groups, and C_1-C_6 straight chain or branched saturated and C_2-C_6 straight chain or branched unsaturated aliphatic hydrocarbon groups substituted with C_3-C_6 cyclic saturated and unsaturated aliphatic hydrocarbon groups having the specified number of carbon atoms. For example, this definition shall include but is not limited to methyl (Me), ethyl (Et), propyl (Pr), butyl (Bu), pentyl, hexyl, heptyl, ethenyl, propenyl, butenyl, penentyl, hexenyl, isopropyl (i-Pr), isobutyl (i-Bu), *tert*-butyl (*t*-Bu), *sec*-butyl (*s*-Bu), isopentyl, neopentyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclopentenyl, cyclohexenyl, methylcyclopropyl, ethylcyclohexenyl, butenylcyclopentyl, and the like.

The term "substituted alkyl" represents an alkyl group as defined above wherein the substitutents are independently selected from halo, cyano, nitro, trihalomethyl, carbamoyl, hydroxy, COR_5, C_1-C_6alkyl, C_1-C_6alkyloxy, aryloxy, arylC_1-C_6alkyloxy, thio, C_1-C_6alkylthio, arylthio, arylC_1-C_6alkylthio, NR_7R_8, C_1-C_6alkylamino, arylamino, arylC_1-C_6alkylamino, di(arylC_1-C_6alkyl)amino, C_1-C_6alkylcarbonyl, arylC_1-C_6alkylcarbonyl, C_1-C_6alkyl-carboxy, arylC_1-C_6alkylcarboxy, C_1-C_6alkylcarbonylamino, -C_1-C_6alkyl-aminoCOR_{11}, arylC_1-C_6alkylcarbonylamino, tetrahydrofuranyl, morpholinyl, piperazinyl, -$CONR_7R_8$, -C_1-C_6alkyl$CONR_7R_8$, or a saturated or partial saturated cyclic 5, 6 or 7 membered amine or lactam; wherein R_{11} is hydroxy, C_1-C_6alkyl, aryl, arylC_1-C_6alkyl, C_1-C_6alkyloxy, aryloxy, arylC_1-C_6alkyloxy and R_5 is defined as above or NR_7R_8, wherein R_7, R_8 are defined as above.

The term "alkyloxy" (e.g. methoxy, ethoxy, propyloxy, allyloxy, cyclohexyloxy) represents an "alkyl" group as defined above having the indicated number of carbon atoms attached through an oxygen bridge. The term "alkyloxyalkyl" represents an "alkyloxy" group attached through an alkyl group as defined above having the indicated number of carbon atoms.

The term "aryloxy" (e.g. phenoxy, naphthyloxy and the like) represents an aryl group as defined below attached through an oxygen bridge.

The term "arylalkyloxy" (e.g. phenethyloxy, naphthylmethyloxy and the like) represents an "arylalkyl" group as defined below attached through an oxygen bridge.

The term "arylalkyloxyalkyl" represents an "arylalkyloxy" group as defined above attached through an "alkyl" group defined above having the indicated number of carbon atoms.

The term "arylthio" (e.g. phenylthio, naphthylthio and the like) represents an "aryl" group as defined below attached through an sulfur bridge.

The term "alkyloxycarbonyl" (e.g. methylformiat, ethylformiat and the like) represents an "alkyloxy" group as defined above attached through a carbonyl group.

The term "aryloxycarbonyl" (e.g. phenylformiat, 2-thiazolylformiat and the like) represents an "aryloxy" group as defined above attached through a carbonyl group.

The term "arylalkyloxycarbonyl" (e.g. benzylformiat, phenyletylformiat and the like) represents an "arylalkyloxy" group as defined above attached through a carbonyl group.

The term "alkyloxycarbonylalkyl" represents an "alkyloxycarbonyl" group as defined above attached through an "alkyl" group as defined above having the indicated number of carbon atoms.

The term "arylalkyloxycarbonylalkyl" represents an "arylalkyloxycarbonyl" group as defined above attached through an "alkyl" group as defined above having the indicated number of carbon atoms.

The term "alkylthio" (e.g. methylthio, ethylthio, propylthio, cyclohexenylthio and the like) represents an "alkyl" group as defined above having the indicated number of carbon atoms attached through a sulfur bridge.

The term "arylalkylthio" (e.g. phenylmethylthio, phenylethylthio, and the like) represents an "arylalkyl" group as defined above having the indicated number of carbon atoms attached through a sulfur bridge.

The term "alkylthioalkyl" represents an "alkylthio" group attached through an alkyl group as defined above having the indicated number of carbon atoms.
The term "arylalkylthioalkyl" represents an "arylalkylthio" group attached through an alkyl group as defined above having the indicated number of carbon atoms.

The term "alkylamino" (e.g. methylamino, diethylamino, butylamino, N-propyl-N-hexylamino, (2-cyclopentyl)propylamino, hexenylamino, pyrrolidinyl, piperidinyl and the like) represents one or two "alkyl" groups as defined above having the indicated number of carbon atoms attached through an amine bridge. The two alkyl groups may be taken together with the nitrogen to which they are attached forming a saturated, partially saturated or aromatic cyclic, bicyclic or tricyclic ring system containing 3 to 14 carbon atoms and 0 to 3 additional heteroatoms selected from nitrogen, oxygen or sulfur, the ring system can optionally be substituted with at least one C_1-C_6alkyl, aryl, arylC_1-C_6alkyl, hydroxy, oxo, C_1-C_6alkyloxy, C_1-C_6alkyloxyC_1-C_6alkyl, NR_9R_{10}, C_1-C_6alkylaminoC_1-C_6alkyl substituent wherein the alkyl and aryl groups are optionally substituted as defined in the definition section and R_9 and R_{10} are defined as above.

The term "arylalkylamino" (e.g. benzylamino, diphenylethylamino and the like) represents one or two "arylalkyl" groups as defined above having the indicated number of carbon atoms attached through an amine bridge. The two "arylalkyl" groups may be taken together with the nitrogen to which they are attached forming a saturated, partially saturated or aromatic cyclic, bicyclic or tricyclic ring system containing 3 to 14 carbon atoms and 0 to 3 additional heteroatoms selected from nitrogen, oxygen or sulfur, the ring system can optionally be substituted with at least one C_1-C_6alkyl, aryl, arylC_1-C_6alkyl, hydroxy, oxo, C_1-C_6alkyloxy, C_1-C_6alkyloxyC_1-C_6alkyl, NR_9R_{10}, C_1-C_6alkylaminoC_1-C_6alkyl substituent wherein the alkyl and aryl groups are optionally substituted as defined in the definition section and R_9 and R_{10} are defined as above.

The term "alkylaminoalkyl" represents an "alkylamino" group attached through an alkyl group as defined above having the indicated number of carbon atoms.

The term "arylalkylaminoalkyl" represents an "arylalkylamino" group attached through an alkyl group as defined above having the indicated number of carbon atoms.

The term "arylalkyl" (e.g. benzyl, phenylethyl) represents an "aryl" group as defined below attached through an alkyl having the indicated number of carbon atoms or substituted alkyl group as defined above.

The term "alkylcarbonyl" (e.g. cyclooctylcarbonyl, pentylcarbonyl, 3-hexenylcarbonyl) represents an "alkyl" group as defined above having the indicated number of carbon atoms attached through a carbonyl group.

The term "arylalkylcarbonyl" (e.g. phenylcyclopropylcarbonyl, phenylethylcarbonyl and the like) represents an "arylalkyl" group as defined above having the indicated number of carbon atoms attached through a carbonyl group.

The term "alkylcarbonylalkyl" represents an "alkylcarbonyl" group attached through an "alkyl" group as defined above having the indicated number of carbon atoms.

The term "arylalkylcarbonylalkyl" represents an "arylalkylcarbonyl" group attached through an alkyl group as defined above having the indicated number of carbon atoms.

The term "alkylcarboxy" (e.g. heptylcarboxy, cyclopropylcarboxy, 3-pentenylcarboxy) represents an "alkylcarbonyl" group as defined above wherein the carbonyl is in turn attached through an oxygen bridge.

The term "arylalkylcarboxy" (e.g. benzylcarboxy, phenylcyclopropylcarboxy and the like) represents an "arylalkylcarbonyl" group as defined above wherein the carbonyl is in turn attached through an oxygen bridge.

The term "alkylcarboxyalkyl" represents an "alkylcarboxy" group attached through an "alkyl" group as defined above having the indicated number of carbon atoms.
The term "arylalkylcarboxyalkyl" represents an "arylalkylcarboxy" group attached through an "alkyl" group as defined above having the indicated number of carbon atoms.

The term "alkylcarbonylamino" (e.g. hexylcarbonylamino, cyclopentylcarbonyl-aminomethyl, methylcarbonylaminophenyl) represents an "alkylcarbonyl" group as defined above wherein the carbonyl is in turn attached through the nitrogen atom of an amino group. The nitrogen atom may itself be substituted with an alkyl or aryl group.

The term "arylalkylcarbonylamino" (e.g. benzylcarbonylamino and the like) represents an "arylalkylcarbonyl" group as defined above wherein the carbonyl is in turn attached through the nitrogen atom of an amino group. The nitrogen atom may itself be substituted with an alkyl or aryl group.

The term "alkylcarbonylaminoalkyl" represents an "alkylcarbonylamino" group attached through an "alkyl" group as defined above having the indicated number of carbon atoms. The nitrogen atom may itself be substituted with an alkyl or aryl group.

The term "arylalkylcarbonylaminoalkyl" represents an "arylalkylcarbonylamino" group attached through an "alkyl" group as defined above having the indicated number of carbon atoms. The nitrogen atom may itself be substituted with an alkyl or aryl group.

The term "alkylcarbonylaminoalkylcarbonyl" represents an alkylcarbonylaminoalkyl group attached through a carbonyl group. The nitrogen atom may be further substituted with an "alkyl" or "aryl" group.

The term "aryl" represents an unsubstituted, mono-, di- or trisubstituted monocyclic, polycyclic, biaryl and heterocyclic aromatic groups covalently attached at any ring position capable of forming a stable covalent bond, certain preferred points of attachment being apparent to those skilled in the art (e.g., 3-indolyl, 4-imidazolyl). The aryl substituents are independently selected from the group consisting of halo, nitro, cyano, trihalomethyl, C_1-C_6alkyl, aryl, arylC_1-C_6alkyl, hydroxy, COR_5, C_1-C_6alkyloxy, C_1-C_6alkyloxyC_1-C_6alkyl, aryloxy, arylC_1-C_6alkyloxy, arylC_1-C_6alkyloxyC_1-C_6alkyl, thio, C_1-C_6alkylthio, C_1-C_6alkylthioC_1-C_6alkyl, arylthio, arylC_1-C_6alkylthio, arylC_1-C_6alkylthioC_1-C_6alkyl, NR_8R_9, C_1-C_6-alkylamino, C_1-C_6alkylaminoC_1-C_6alkyl, arylamino, arylC_1-C_6alkylamino, arylC_1-C_6alkylaminoC_1-C_6alkyl, di(arylC_1-C_6alkyl)-aminoC_1-C_6alkyl, C_1-C_6alkylcarbonyl, C_1-C_6alkylcarbonylC_1-C_6alkyl, arylC_1-C_6alkyl-carbonyl, arylC_1-C_6alkylcarbonylC_1-C_6alkyl, C_1-C_6alkylcarboxy, C_1-C_6alkylcarboxyC_1-C_6alkyl, arylC_1-

C_6alkylcarboxy, arylC_1-C_6alkylcarboxyC_1-C_6alkyl, carboxyC_1-C_6alkyloxy, C_1-C_6alkylcarbonylamino, C_1-C_6alkylcarbonylaminoC_1-C_6alkyl, -carbonylNR_7C_1-C_6alkylCOR_{11}, arylC_1-C_6alkylcarbonyl-amino, arylC_1-C_6alkylcarbonylaminoC_1-C_6alkyl, -$CONR_8R_9$, or -C_1-C_6alkyl-$CONR_8R_9$; wherein R_7, R_8, R_9, and R_{11} are defined as above and the alkyl and aryl groups are optionally substituted as defined in the definition section;

The definition of aryl includes but is not limited to phenyl, biphenyl, indenyl, fluorenyl, naphthyl (1-naphthyl, 2-naphthyl), pyrrolyl (2-pyrrolyl), pyrazolyl (3-pyrazolyl), imidazolyl (1-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), triazolyl (1,2,3-triazol-1-yl, 1,2,3-triazol-2-yl 1,2,3-triazol-4-yl, 1,2,4-triazol-3-yl), oxazolyl (2-oxazolyl, 4-oxazolyl, 5-oxazolyl), isoxazolyl (3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl), thiazolyl (2-thiazolyl, 4-thiazolyl, 5-thiazolyl), thiophenyl, (2-thiophenyl, 3-thiophenyl, 4-thiophenyl, 5-thiophenyl), furanyl (2-furanyl, 3-furanyl, 4-furanyl, 5-furanyl), pyridyl (2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl), pyrimidinyl (2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl), pyrazinyl, pyridazinyl (3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl), quinolyl (2-quinolyl, 3-quinolyl, 4-quinolyl, 5-quinolyl, 6-quinolyl, 7-quinolyl, 8-quinolyl), isoquinolyl (1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 5-isoquinolyl, 6-isoquinolyl, 7-isoquinolyl, 8-isoquinolyl), benzo[b]furanyl (2-benzo[b]furanyl, 3-benzo[b]furanyl, 4-benzo[b]furanyl, 5-benzo[b]furanyl, 6-benzo[b]furanyl, 7-benzo[b]furanyl), 2,3-dihydro-benzo[b]furanyl (2-(2,3-dihydro-benzo[b]furanyl), 3-(2,3-dihydro-benzo[b]furanyl), 4-(2,3-dihydro-benzo[b]furanyl), 5-(2,3-dihydro-benzo[b]furanyl), 6-(2,3-dihydro-benzo[b]furanyl), 7-(2,3-dihydro-benzo[b]furanyl)), benzo[b]thiophenyl (2-benzo[b]thiophenyl, 3-benzo[b]thiophenyl, 4-benzo[b]thiophenyl, 5-benzo[b]thiophenyl, 6-benzo[b]thiophenyl, 7-benzo[b]thiophenyl), 2,3-dihydro-benzo[b]-thiophenyl (2-(2,3-dihydro-benzo[b]thiophenyl), 3-(2,3-dihydro-benzo[b]-thiophenyl), 4-(2,3-dihydro-benzo[b]thiophenyl), 5-(2,3-dihydro-benzo[b]-thiophenyl), 6-(2,3-dihydro-benzo[b]thiophenyl), 7-(2,3-dihydro-benzo[b]-thiophenyl)), 4,5,6,7-tetrahydro-benzo[b]thiophenyl (2-(4,5,6,7-tetrahydro-benzo[b]thiophenyl), 3-(4,5,6,7-tetrahydro-benzo-[b]thiophenyl), 4-(4,5,6,7-tetrahydro-benzo[b]thiophenyl), 5-(4,5,6,7-tetrahydro-benzo-[b]thiophenyl), 6-(4,5,6,7-tetrahydro-benzo[b]thiophenyl), 7-(4,5,6,7-tetrahydro-benzo[b]thiophenyl)), 4,5,6,7-tetrahydro-thieno[2,3-c]pyridyl (4-(4,5,6,7-tetrahydro-thieno[2,3-c]pyridyl), 5-4,5,6,7-tetrahydro-thieno[2,3-c]pyridyl), 6-(4,5,6,7-tetrahydro-thieno[2,3-c]pyridyl), 7-(4,5,6,7-tetra-hydro-thieno[2,3-c]pyridyl)), indolyl (1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), indazole (1-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl (1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-

benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (1-benzoxazolyl, 2-benzoxazolyl), benzothiazolyl (1-benzothiazolyl, 2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl, 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl), 5H-dibenz[b,f]azepine (5H-dibenz[b,f]azepin-1-yl, 5H-dibenz[b,f]azepine-2-yl, 5H-dibenz[b,f]azepine-3-yl, 5H-dibenz-[b,f]azepine-4-yl, 5H-dibenz[b,f]-azepine-5-yl), 10,11-dihydro-5H-dibenz[b,f]azepine (10,11-dihydro-5H-dibenz[b,f]azepine-1-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-2-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-3-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-4-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-5-yl), piperidinyl (2-piperidinyl, 3-piperidinyl, 4-piperidinyl), pyrrolidinyl (1-pyrrolidinyl, 2-pyrrolidinyl, 3-pyrrolidinyl), phenylpyridyl (2-phenyl-pyridyl, 3-phenylpyridyl, 4-phenylpyridyl), phenylpyrimidinyl (2-phenylpyrimidinyl, 4-phenylpyrimidinyl, 5-phenylpyrimidinyl, 6-phenylpyrimidinyl), phenylpyrazinyl, phenylpyridazinyl (3-phenylpyridazinyl, 4-phenylpyridazinyl, 5-phenyl-pyridazinyl).

The term "arylcarbonyl" (e.g. 2-thiophenylcarbonyl, 3-methoxy-anthrylcarbonyl, oxazolylcarbonyl) represents an "aryl" group as defined above attached through a carbonyl group.

The term "arylalkylcarbonyl" (e.g. (2,3-dimethoxyphenyl)-propylcarbonyl, (2-chloronaphthyl)pentenylcarbonyl, imidazolylcyclo-pentylcarbonyl) represents an "arylalkyl" group as defined above wherein the "alkyl" group is in turn attached through a carbonyl.

The compounds of the present invention which have asymmetric centers may occur as racemates, racemic mixtures, and as individual enantiomers or diastereoisomers, with all isomeric forms being included in the present invention as well as mixtures thereof.

Pharmaceutically acceptable salts of the compounds of the invention, where a basic or acidic group is present in the structure, are also included within the scope of this invention. When an acidic substituent is present, such as -COOH, 5-tetrazolyl and $P(O)(OH)_2$, there can be formed the ammonium, sodium, potassium, calcium salt, and the like, for use as the dosage form. When a basic group is present, such as amino or a basic heteroaryl radical, such as pyridyl, an acidic salt, such as hydrochloride, hydrobromide, acetate, maleate, palmoate, methanesulfonate, p-toluenesulfonate, and the like, can be used as the dosage form.

Also, in the case of the -COOH or $-P(O)(OH)_2$ being present, pharmaceutically acceptable esters can be employed, e.g., methyl, tert-butyl, pivaloyloxymethyl, and the like, and those esters known in the art for modifying solubility or hydrolysis characteristics for use as sustained release or prodrug formulations.

In addition, some of the compounds of the instant invention may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of the invention.

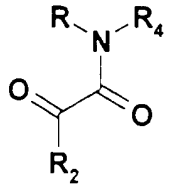
The term "therapeutically effective amount" shall mean that amount of drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal, or human that is being sought by a researcher, veterinarian, medical doctor or other.

DESCRIPTION OF THE INVENTION

It has surprisingly been shown that compounds comprising a certain structural fragment show inhibitory or modulatory capacity against one or more PTPases or other molecules with phosphotyrosine recognition unit(s).

Accordingly, the present invention relates to compounds that fulfills all of the following 3 criteria:

(1) has a structure represented by Formula I:



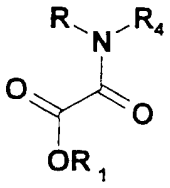
Formula I

wherein R, R_2 and R_4 are any chemical group or combination of chemical groups;

(2) acts as a phosphotyrosine recognition unit ligand, preferably an inhibitor or modulator of one or more PTPases or proteins that contain SH2 domains; and

(3) has a molecular weight below or equal to 2500 daltons;

In a preferred embodiment the compounds of the invention has a structure represented by Formula II

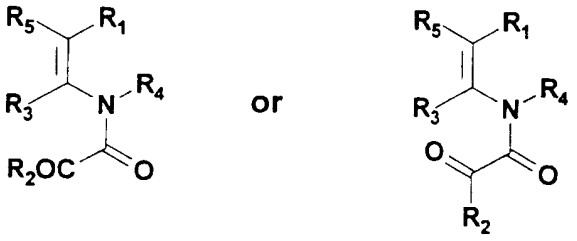


Formula II

where R, $R_1$and R_4 are any chemical group or combination of chemical groups, and R_1 preferably is H.

In another preferred embodiment the compounds that fulfills all of the following 3 criteria:

(1) has a structure represented by Formula III:



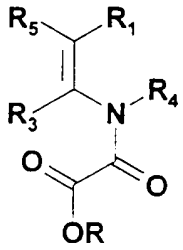
Formula III

wherein R_1, R_2, R_3, R_4 and R_5 are any chemical group or combination of chemical groups, and R_3 and R_5 may be covalently linked to each other;

(2) acts as a phosphotyrosine recognition unit ligand, preferably an inhibitor or modulator of one or more PTPases or proteins that contain SH2 domains; and

(3) has a molecular weight below or equal to 2500 daltons.

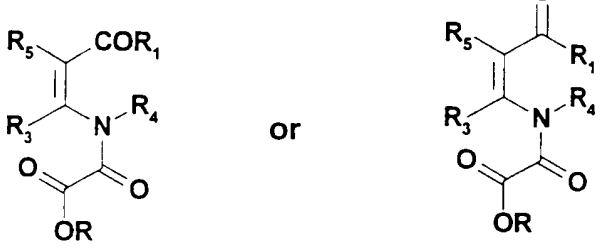
In another preferred embodiment the compounds of the invention has a structure represented by Formula IV



Formula IV

where R_1, R_3, R_4 and R_5 are any chemical group or combination of chemical groups, and R_3 and R_5 may be covalently linked to each other, and R preferably is H.

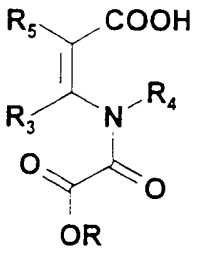
In another preferred embodiment the compounds of the invention has a structure represented by Formula V



Formula V

where R_1, R_3, R_4 and R_5 are any chemical group or combination of chemical groups, and R_3 and R_5 may be covalently linked to each other, and R is preferably H.

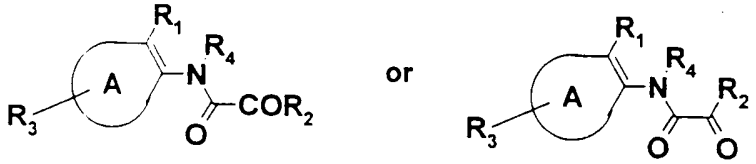
In another preferred embodiment the compounds of the invention has a structure represented by Formula VI



Formula VI

where R_3, R_4 and R_5 are any chemical group or combination of chemical groups, and R_3 and R_5 may be covalently linked to each other, and R is preferably H.

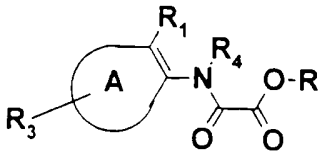
In another preferred embodiment the compounds of the invention has a structure represented by Formula VII



Formula VII

wherein A together with the double bond in formula VII represents any aryl as defined above, and R_1, R_2, R_3 and R_4 are any chemical group or combination of chemical groups.

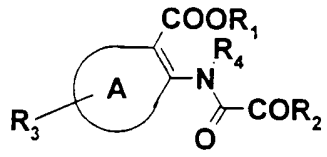
In another preferred embodiment the compounds of the invention has a structure represented by Formula VIII



Formula VIII

wherein A together with the double bond in formula VIII represents any aryl as defined above, and R, R_1, R_3 and R_4 are any chemical group or combination of chemical groups, and R preferably is H.

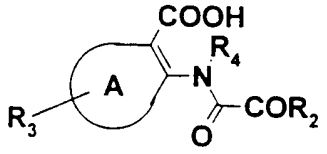
In another preferred embodiment the compounds of the invention has a structure represented by Formula IX



Formula IX

wherein A together with the double bond in formula IX represents any aryl as defined above, and R_1, R_2, R_3 and R_4 are any chemical group or combination of chemical groups.

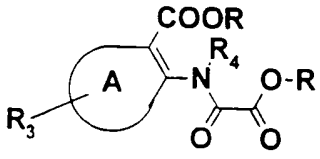
In another preferred embodiment the compounds of the invention has a structure represented by Formula X



Formula X

wherein A together with the double bond in formula X represents any aryl as defined above, and R_2, R_3 and R_4 are any chemical group or combination of chemical groups.

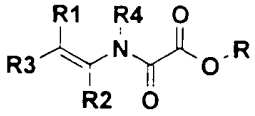
In another preferred embodiment the compounds of the invention has a structure represented by Formula XI



Formula XI

wherein A together with the double bond in formula XI represents any aryl as defined above, and R, R_3 and R_4 are any chemical group or combination of chemical groups, and R preferably is H

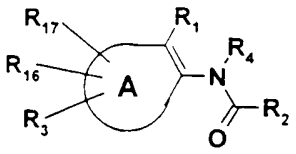
In another preferred embodiment the compounds of the invention has a structure represented by Formula XII



Formula XII

wherein R_1 is a chemical group capable of being a proton donor and/or a proton acceptor, preferably -COOH, 5-tetrazolyl, $-NH_2$, $-CONH_2$, and R, R_2, R_3 and R_4 are any chemical group or combination of chemical groups.

In another preferred embodiment the structure of the compounds of the invention is represented by the following Formula XX



Formula XX

wherein:

A is together with the double bond in Formula 1 phenyl, biphenyl, indenyl, fluorenyl, fluorenyl-9-one, naphthyl, pyridyl, pyridazinyl, pyrimidyl or pyrazinyl;

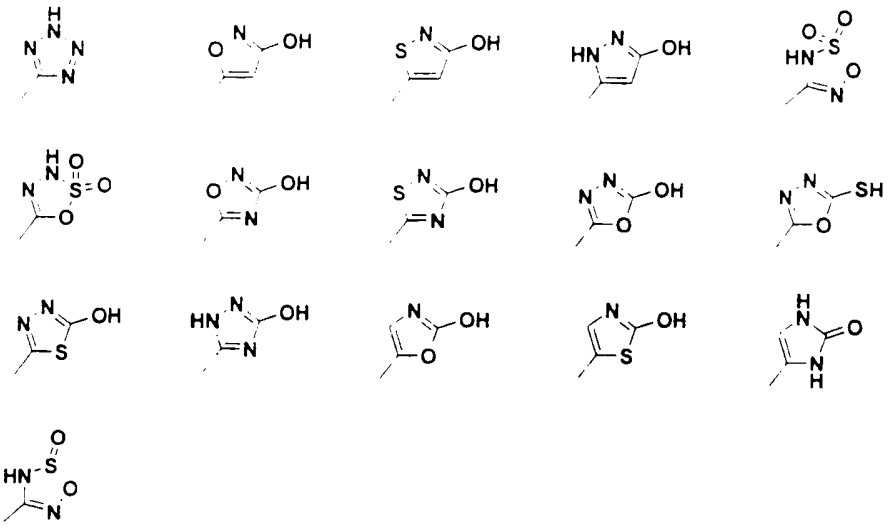
or A is together with the double bond in Formula 1 indolyl, benzo[b]thiophenyl, benzo[b]furanyl, indazolyl, benzo[b]isoxazolyl, benzimidazolyl, benzthiazolyl, benzoxazolyl, 9H-thieno[2,3-c]chromenyl, 4,5,6,7-tetrahydro-benzo[b]thiophenyl, 4,5,6,7-tetrahydro-

thieno[2,3-b]pyridyl, 4,5,6,7-tetrahydro-thieno[2,3-c]pyridyl, 4,5,6,7-tetrahydro-thieno[3,2-c]pyridyl, 4,5,6,7-tetrahydro-thieno[3,2-b]pyridyl, 4,7-dihydro-5H-thieno[2,3-c]pyranyl, 4,7-dihydro-5H-thieno[2,3-c]thiopyranyl or 4,5,6,7-tetrahydro-4,7-ethanon-thieno[2,3-b]pyridyl;

or A is together with the double bond in Formula 1 furanyl, thiophenyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, 1,2,3-oxadiazolyl, furazanyl or 1,2,3-triazolyl;

or A is together with the double bond in Formula 1 furo[2,3-b]pyridyl, thieno[2,3-b]pyridyl, pyrrolo[2,3-b]pyridyl, furo[2,3-c]pyridyl, thieno[2,3-c]pyridyl, pyrrolo[2,3-c]pyridyl, furo[3,2-c]pyridyl, thieno[3,2-c]pyridyl, pyrrolo[3,2-c]pyridyl, furo[3,2-d]pyridyl, thieno[3,2-d]pyridyl, pyrrolo[3,2-d]pyridyl, furo[2,3-d]pyrimidyl, thieno[2,3-d]pyrimidyl, pyrrolo[2,3-d]pyrimidyl, furo[2,3-b]pyrazinyl, thieno[2,3-b]pyrazinyl, pyrrolo[2,3-b]pyrazinyl, furo[2,3-c]pyridazinyl, thieno[2,3-c]pyridazinyl, pyrrolo[2,3-c]pyridazinyl, furo[2,3-d]pyridazinyl, thieno[2,3-d]pyridazinyl, pyrrolo[2,3-d]pyridazinyl, furo[3,2-c]pyridazinyl, thieno[3,2-c]pyridazinyl, pyrrolo[3,2-c]pyridazinyl, quinolizinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, chromanyl, thiochromanyl, isochromanyl, isothiochromanyl, 2,3-dihydro-thieno[2,3-b]furanyl, 4,6-dihydro-thieno[2,3-c]furanyl, 2,3-dihydro-thieno[3,2-b]furanyl, 4,5-dihydro-thieno[2,3-b]thiophenyl, 4,6-dihydro-thieno[3,4-b]thiophenyl, 5,6-dihydro-thieno[3,2-b]thiophenyl, 4,5-dihydro-thieno[2,3-b]pyrrolyl, thieno[3,2-d]isothiazolyl, thieno[3,2-d]thiazolyl, thieno[2,3-d]thiazolyl, thieno[2,3-c]pyrrolyl-4,6-dione, 1H-thieno[2,3-d]imidazolyl, 6H-thieno[2,3-b]pyrrolyl, 5,6-dihydro-4H-thieno[2,3-c]pyrrolyl or 4H-thieno[3,2-b]pyrrolyl;

R_1 and R_2 are independently selected from the group consisting of COR_5, OR_6, CF_3, nitro, cyano, SO_3H, $SO_2NR_7R_8$, $PO(OH)_2$, $CH_2PO(OH)_2$, $CHFPO(OH)_2$, $CF_2PO(OH)_2$, $C(=NH)NH_2$, NR_7R_8, and the following 5-membered heterocycles



R_3, R_{16} and R_{17} are hydrogen, halo, nitro, cyano, trihalomethyl, C_1-C_6alkyl, aryl, arylC_1-C_6-alkyl, hydroxy, carboxy, carboxyC_1-C_6alkyl, C_1-C_6alkyloxycarbonyl, aryloxycarbonyl, arylC_1-C_6alkyloxycarbonyl, C_1-C_6alkyloxy, C_1-C_6alkyloxyC_1-C_6alkyl, aryloxy, arylC_1-C_6alkyloxy, arylC_1-C_6alkyloxyC_1-C_6alkyl, thio, C_1-C_6alkylthio, C_1-C_6alkylthioC_1-C_6alkyl, arylthio, arylC_1-C_6alkylthio, arylC_1-C_6alkylthioC_1-C_6alkyl, NR_7R_8, C_1-C_6alkyl-aminoC_1-C_6alkyl, arylC_1-C_6alkylaminoC_1-C_6alkyl, di(arylC_1-C_6alkyl)aminoC_1-C_6alkyl, C_1-C_6alkylcarbonyl, C_1-C_6alkylcarbonylC_1-C_6alkyl, arylC_1-C_6alkylcarbonyl, arylC_1-C_6alkylcarbonylC_1-C_6alkyl, C_1-C_6alkylcarboxy, C_1-C_6alkylcarboxyC_1-C_6-alkyl, arylcarboxy, arylC_1-C_6alkylcarboxy, arylC_1-C_6alkylcarboxyC_1-C_6alkyl, C_1-C_6alkylcarbonyl-amino, C_1-C_6alkylcarbonylaminoC_1-C_6alkyl, -carbonylNR_7C_1-C_6alkylCOR_{11}, arylC_1-C_6alkylcarbonylamino, arylC_1-C_6alkylcarbonylaminoC_1-C_6alkyl, $CONR_7R_8$, or C_1-C_6alkyl$CONR_7R_8$ wherein the alkyl and aryl groups are optionally substituted and R_{11} is NR_7R_8, or C_1-C_6alkylNR_7R_8; or, when R_{16} and R_{17} are hydrogen, R_3 is

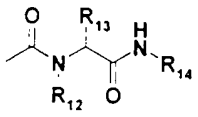
A-B-C-D-C_1-C_6alkyl, wherein

A is C_1-C_8alkyl, aryl or arylC_1-C_6alkyl;

B is amino, thio, SO, SO_2 or oxo;

C is C_1-C_8alkyl, amino;

D is a chemical bond, amino or C_1-C_8alkyl wherein the alkyl and aryl groups are optionally substituted; or



wherein R_{12}, R_{13}, and R_{14} are independently hydrogen, C_1-C_6alkyl, aryl, arylC_1-C_6alkyl and the alkyl and aryl groups are optionally substituted;

R_4 is hydrogen, hydroxy, C_1-C_6alkyl, aryl, arylC_1-C_6alkyl, NR_7R_8, C_1-C_6alkyloxy; wherein the alkyl and aryl groups are optionally substituted;

R_5 is hydroxy, C_1-C_6alkyl, aryl, arylC_1-C_6alkyl, CF_3, NR_7R_8; wherein the alkyl and aryl groups are optionally substituted;

R_6 is hydrogen, C_1-C_6alkyl, aryl, arylC_1-C_6alkyl; wherein the alkyl and aryl groups are optionally substituted;

R_7 and R_8 are independently selected from hydrogen, C_1-C_6alkyl, aryl, arylC_1-C_6alkyl, C_1-C_6alkylcarbonyl, arylcarbonyl, arylC_1-C_6alkylcarbonyl, C_1-C_6alkyl-carboxy or arylC_1-C_6alkylcarboxy wherein the alkyl and aryl groups are optionally substituted; or
R_7 and R_8 are together with the nitrogen to which they are attached forming a saturated, partially saturated or aromatic cyclic, bicyclic or tricyclic ring system containing from 3 to 14 carbon atoms and from 0 to 3 additional heteroatoms selected from nitrogen, oxygen or sulfur, the ring system can optionally be substituted with at least one C_1-C_6alkyl, aryl, arylC_1-C_6alkyl, hydroxy, oxo, C_1-C_6alkyloxy, arylC_1-C_6alkyloxy, C_1-C_6alkyloxyC_1-C_6alkyl, NR_9R_{10} or C_1-C_6alkylaminoC_1-C_6alkyl, wherein R_9 and R_{10} are independently selected from hydrogen, C_1-C_6alkyl, aryl, arylC_1-C_6alkyl, C_1-C_6alkylcarbonyl, arylcarbonyl, arylC_1-C_6alkylcarbonyl, C_1-C_6alkylcarboxy or arylC_1-C_6alkylcarboxy; wherein the alkyl and aryl groups are optionally substituted; or
R_7 and R_8 are independently a saturated or partial saturated cyclic 5, 6 or 7 membered amine, imide or lactam;

Compounds of the invention may modulate or inhibit the activity of protein tyrosine phosphatases or other molecules with phosphotyrosine recognition unit(s) via different mechanisms of action. Examples of such mechanism of actions, which are not intended in any way to limit the scope of the invention, are (a) classical competitive inhibition; (b) uncompetitive inhibition; (c) mixed-type inhibition as defined above.

The invention furthermore relates to compounds which after uptake in cells or mammals has a structure as defined above.

In one preferred embodiment, the compounds of the invention act as classical, competitive inhibitors of one or more PTPases.

In another preferred embodiment, the compounds of the invention act as mixed-type inhibitors of one or more PTPases.

In one preferred embodiment, the compounds of the invention substantially act as an inhibitor of one or more PTPases involved in regulation of tyrosine kinase signalling pathways.

In another preferred embodiment, the compounds of the invention substantially inhibit or modulate receptor-tyrosine kinase signalling pathways via interaction with one or more regulatory PTPases, preferably the signalling pathways of the insulin receptor, the IGF-I receptor and/or other members of the insulin receptor family, the EGF-receptor family, the platelet-derived growth factor receptor family, the nerve growth factor receptor family, the hepatocyte growth factor receptor family, the growth hormone receptor family and/or members of other receptor-type tyrosine kinase families.

In another preferred embodiment, the compounds of the invention substantially inhibit or modulate non-receptor tyrosine kinase signalling through modulation of one or more regulatory PTPases, preferably modulation of members of the Src kinase family or other intracellular kinases.

In another preferred embodiment, the compounds of the invention substantially inhibit or modulate the activity of one or more PTPases that negatively regulate signal transduction pathways.

In another preferred embodiment, the compounds of the invention inhibit or modulate the activity of one or more PTPases that positively regulate signal transduction pathways, preferably CD45.

In another preferred embodiment, the compounds of the invention inhibit or modulate the activity of one or more PTPases that positively regulate signal transduction pathways in immune cells.

In another preferred embodiment, the compounds of the invention inhibit or modulate the activity of one or more PTPases that negatively regulate signal transduction pathway.

In another preferred embodiment, the compounds of the invention inhibit one or more PTPases via binding to the active site of said PTPase(s) or to other sites that negatively influences the binding of substrate to said PTPase(s), an allosteric modulator.

In another preferred embodiment, the compounds of the invention modulate the activity of one or more PTPases via interaction with structures positioned outside of the active sites of the enzymes, preferably SH2 domains.

In another preferred embodiment, the compounds of the invention modulate the signal transduction pathways via binding of the compounds of the invention to SH2 domains or PTB domains of non-PTPase signalling molecules.

In one embodiment, the compounds of the invention are characterized by being selective PTPase inhibitors or compounds that are selective phosphotyrosine recognition unit ligands. The compound of the invention can e.g. be selective for a PTPase not described herein or, preferably, a PTPase listed in Table 1.

In another preferred embodiment, the compounds of the invention are characterized by being non-selective PTPase inhibitors such as inhibitors or modulators of at least 4 PTPases or 4 PTPase families.

In one preferred embodiment, the compounds of the invention are selective for the PTPα family.

In another preferred embodiment, the compounds of the invention are selective for PTPα.

In another preferred embodiment, the compounds of the invention are selective for PTPε.

In another preferred embodiment, the compounds of the invention are selective for CD45.

In one preferred embodiment, the compounds of the invention are selective for PTPβ family.

In another preferred embodiment, the compounds of the invention are selective for PTPβ.

In another preferred embodiment, the compounds of the invention are selective for PTP-DEP1.

In one preferred embodiment, the compounds of the invention are selective for PTP-LAR family.

In one preferred embodiment, the compounds of the invention are selective for PTP-LAR.

In one preferred embodiment, the compounds of the invention are selective for PTPσ.

In one preferred embodiment, the compounds of the invention are selective for PTPδ.

In one preferred embodiment, the compounds of the invention are selective for PTPμ family.

In one preferred embodiment, the compounds of the invention are selective for PTPμ.

In one preferred embodiment, the compounds of the invention are selective for PTPκ.

In one preferred embodiment, the compounds of the invention are selective for PTP1B family.

In one preferred embodiment, the compounds of the invention are selective for PTP1B.

In one preferred embodiment, the compounds of the invention are selective for TC-PTP.

In one preferred embodiment, the compounds of the invention are selective for SHP-PTP family.

In one preferred embodiment, the compounds of the invention are selective for SHP-1.

In one preferred embodiment, the compounds of the invention are selective for SHP-2.

In one preferred embodiment, the compounds of the invention are selective for PTPζ family.

In another preferred embodiment, the compounds of the invention are selective for PTPγ.

In one preferred embodiment, the compounds of the invention are selective for PTP-PEST family.

In one preferred embodiment, the compounds of the invention are selective for PTPH1 family.

In one preferred embodiment, the compounds of the invention are selective for PTPH1.

In one preferred embodiment, the compounds of the invention are selective for PTPD1.

In one preferred embodiment, the compounds of the invention are selective for PTPD2.

In one preferred embodiment, the compounds of the invention are selective for PTPMEG1.

In one preferred embodiment, the compounds of the invention are selective for IA-2 family.

In one preferred embodiment, the compounds of the invention are selective for IA-2.

In one preferred embodiment, the compounds of the invention are selective for IA-2β.

In one preferred embodiment, the compounds of the invention are selective for the PTPψ family.

In another preferred embodiment, the compounds of the invention are selective for PTPψ.

In another preferred embodiment, the compounds of the invention are selective for PTPρ.

In another preferred embodiment, the compounds of the invention are selective for PTPϕ.

In another preferred embodiment, the compounds of the invention have a molecular weight of less than 1000 Daltons, and preferably of more than 100 Daltons.

In one preferred embodiment, the compounds of the invention have K_i values of less than 200 μM against one or more PTPases.

In another preferred embodiment, the compounds of the invention have K_i values of less than 2 μM against one or more PTPases.

In another preferred embodiment, the compounds of the invention have K_i values of less than 100 nM against one or more PTPases.

In another preferred embodiment, the compounds of the invention have a K_i value of < 2 μM against one or two PTPase or PTPase families and a K_i value of > 50 μM against at least two other PTPases or PTPase families.

In another preferred embodiment, the compounds of the invention have a K_i value of < 100 nM against one or two PTPase or PTPase families and a K_i value of > 10 μM against at least two other PTPases or PTPase families.

In one preferred embodiment, the compounds of the invention have a IC_{50} value of less than 200 M against one or more molecules with phosphotyrosine recognition unit(s).

In another preferred embodiment, the compounds of the invention have a IC_{50} value of less than 2 M against one or more molecules with phosphotyrosine recognition unit(s).

In another preferred embodiment, the compounds of the invention have a IC_{50} value of less than 100 nM against one or more molecules with phosphotyrosine recognition unit(s).

In one preferred embodiment, the compounds of the invention act as inhibitors of one or more PTPases, e.g. protein tyrosine phosphatases involved in regulation of tyrosine kinase signalling pathways. Preferred embodiments include modulation of receptor-tyrosine kinase signalling pathways via interaction with regulatory PTPases, e.g. the signalling pathways of the insulin receptor, the IGF-I receptor and other members of the insulin receptor family, the EGF-receptor family, the platelet-derived growth factor receptor family, the nerve growth factor receptor family, the hepatocyte growth factor receptor family, the growth hormone receptor family and members of other receptor-type tyrosine kinase families. Further preferred embodiments of the inventions is modulation of non-receptor tyrosine kinase signalling through modulation of regulatory PTPases, e.g. modulation of members of the Src kinase family and other non-receptor tyrosine kinases. One type of preferred embodiment of the inventions relates to modulation of the activity of PTPases that negatively regulate signal transduction pathways. An example, which is not intended in any way to limit the scope of the invention, is SHP-1 that negatively regulates

the erythropoietin signalling pathway. Another type of preferred embodiments of the inventions relate to modulation of the activity of PTPases that positively regulate signal transduction pathways. An example of the latter, which is not intended in any way to limit the scope of the invention, is CD45 which dephosphorylates tyrosine kinase of the Src family and thereby plays a positive role in signalling in cells from the hematopoietic system. One type of preferred CD45 inhibitor can be used to regulate the activity of lymphocytes, including T- and/or B-lymphocytes.

In a preferred embodiment the compounds of the invention act as modulators or inhibitors of the active site of one or more PTPases. In another preferred embodiment the compounds of the invention modulate the activity of one or more PTPases via interaction with structures positioned outside of the active sites of the enzymes, preferably SH2 domains. Further preferred embodiments include modulation of signal transduction pathways via binding of the compounds of the invention to SH2 domains or PTB domains of non-PTPase signalling molecules.

In preferred embodiments the compounds of the invention are selective inhibitors that are more than 10-fold potent against one PTPase family than against another PTP family.

In one embodiment, the compounds of the invention can be used for managing, treating or preventing type I diabetes, type II diabetes, impaired glucose tolerance, insulin resistance, obesity, immune dysfunctions including autoimmunity and AIDS, diseases with dysfunctions of the coagulation system, allergic diseases, osteoporosis, proliferative disorders including cancer and psoriasis, diseases with decreased or increased synthesis or effects of growth hormone, diseases with decreased or increased synthesis of hormones or cytokines that regulate the release of/or response to growth hormone, diseases of the brain including Alzheimer's disease and schizophrenia, and infectious diseases.

In another embodiment, the compounds of the invention can be used for

In another embodiment, the compounds of the invention can be used for managing, treating or preventing type I diabetes, type II diabetes, impaired glucose tolerance, insulin resistance, and/or obesity.

In another embodiment, the compounds of the invention can be used for managing, treating or preventing conditions with immune dysfunctions, including autoimmunity such as rheumatoid arthritis, systemic lupus erythematosus.

In another embodiment, the compounds of the invention can be used as immunosuppressants.

In another embodiment, the compounds of the invention can be used for managing or treating conditions with immune dysfunctions including AIDS.

In another embodiment, the compounds of the invention can be used for managing, treating or preventing allergic diseases, including asthma and allergic skin diseases.

In another embodiment, the compounds of the invention can be used for managing, treating or preventing proliferative disorders, including cancer.

In another embodiment, the compounds of the invention can be used for managing, treating or preventing osteoporosis.

In another embodiment, the compounds of the invention can be used for managing, treating or preventing psoriasis.

In another embodiment, the compounds of the invention can be used for managing, treating or preventing diseases with decreased or increased synthesis or effects of growth hormone, diseases with decreased or increased synthesis of hormones or cytokines that regulate the release of/or response to growth hormone.

In another embodiment, the compounds of the invention can be used for managing, treating or preventing diseases with dysfunctions of the coagulation system.

In another embodiment, the compounds of the invention can be used for managing, treating or preventing diseases of the brain including Alzheimer's disease and schizophrenia.

In another embodiment, the compounds of the invention can be used for managing, treating or preventing infectious diseases.

The compounds of the invention can furthermore be used to manufacture of medicaments for managing, treating or preventing the above-mentioned diseases and disorders.

Other preferred embodiments include use of the compounds of the invention for modulation of cell-cell interactions as well as cell-matrix interactions.

The present invention is furthermore concerned with pharmaceutical compositions comprising, as an active ingredient, at least one of the compounds of the present invention in association with a pharmaceutical carrier or diluent. Optionally, the pharmaceutical composition can comprise at least one of the compounds of the invention combined with compounds exhibiting a different activity, e.g. an antibiotic or other pharmacologically active material.

As a preferred embodiment, the compounds of the invention may be used as therapeuticals to inhibit or modulate one or more PTPases involved in regulation of the insulin receptor tyrosine kinase signalling pathway in patients with type I diabetes, type II diabetes, impaired glucose tolerance, insulin resistance, and obesity. Further preferred embodiments include use of the compounds of the invention for management of disorders with general or specific dysfunctions of PTPase activity, e.g. proliferarive disorders such as psoriasis and neoplastic diseases. As another embodiment, the compounds of the invention may be used in pharmaceutical preparations for management of osteoporosis.

Preferred embodiments of the invention further include use of compound of the invention in pharmaceutical preparations to increase the secretion or action of growth hormone and its analogous or somatomedins including IGF-1 and IGF-2 by modulating the activity of one or more PTPases or other signal transduction molecules with affinity for phosphotyrosine involved controlling or inducing the action of these hormones or any regulating molecule.

The compounds of the invention may be used in pharmaceutical preparations for management of various disorders of the immune system, either as a stimulant or suppressor

of normal or perturbed immune functions, including autoimmune reactions. Further embodiments of the invention include use of the compounds of the invention for management of allergic reactions, e.g. asthma, dermal reactions, conjunctivitis.

In another embodiment, the compounds of the invention may be used in pharmaceutical preparations used for immunosuppression. A non-limiting example of such use is in connection with management of organ and/or tissue transplantation.

In another embodiment, compounds of the invention may be used in pharmaceutical preparations for prevention or induction of platelet aggregation.

In yet another embodiment, compounds of the invention may be used in pharmaceutical preparations for management of infectious disorders. In particular, the compounds of the invention may be used for management of infectious disorders caused by *Yersinia* and other bacteria as well as disorders caused by viruses or other micro-organisms.

Compounds of the invention may additionally be used for management or prevention of diseases in animals, including commercially important animals.

Also included in the present invention is a process for isolation of PTPases via affinity purification procedures based on the use of immobilized compounds of the invention using procedures well-known to those skilled in the art. Such methods, well-known to those skilled in the arts, may be used to identify novel PTPases or other molecules with phosphotyrosine recognition units. As a non-limiting example, compounds of the invention may be immobilized by coupling to a solid-phase. A tissue sample or a sample from a cell line prepared as a lysate by methods well-known to those skilled in the art may be passed over said solid-phase coupled with a compound of the invention. After appropriate washing procedures designed to remove material that binds unspecifically to said solid-phase, using standard procedures well known to those skilled in the art, mostly PTPases or other molecules with phosphotyrosine recognition units will be bound to the compounds of the invention coupled to the solid phase. Said PTPases or other molecules with phosphotyrosine recognition units may in turn be released by procedures well-known in the art and further subjected to amino acid sequence analysis according to standard procedures well-known to those skilled in the art. By back-translation of said amino acid sequence into a

nucleotide sequence of the corresponding cDNA can be deduced using the appropriate genetic code. Said nucleotide sequence can be used to design and produce an equivalent oligonucleotide, which in turn can be used to identify partial or full-length cDNA clones from appropriate cDNA libraries encoding a protein or glycoprotein corresponding to or similar to the isolated PTPase or molecule with pTyr recognition units. Said oligonucleotide or isolated cDNA clone(s) can similarly be used to isolate genomic clones corresponding to said cDNA clones. Said partial or full-length cDNA can be inserted into appropriate vectors and expressed and purified proteins with procedures well known to those skilled in the arts. Said purified proteins, in particular PTPases, may be used to further analyze the inhibitory capacity and selectivity of compounds of the invention as described.

The invention is further directed to compounds of the invention coupled to a suitable solid-phase matrix such as a Wang-resin or a Rink-resin

The invention is further diected to a method for isolating a protein or a glycoprotein with affinity for a compound according to the invention from a biological sample, comprising:

- contacting a compound of the invention immobilized by coupling to a suitable solid-phase matrix with said biological sample in order for said immobilized compound to form a complex by binding said protein or glycoprotein,
- removing unbound material from said biological sample and isolating said complex, and
- extracting said protein or glycoprotein from said complex.

The invention is further directed to a method for isolating a protein-tyrosine phosphatase with affinity for a compound according to the invention from a biological sample, comprising

- contacting a compound of the invention immobilized by coupling to a suitable solid-phase matrix with said biological sample in order for said immobilized compound to form a complex by binding said protein-tyrosine phosphatase
- removing unbound material from said biological sample and isolating said complex
- extracting said protein-tyrosine phosphatase.

The invention is further directed to a method for isolating a Src-homology 2 domain containing protein or a phosphotyrosine binding domain containing protein with affinity for a compound of the present invention from a biological sample, comprising

- contacting a compound of the invention immobilized by coupling to a suitable solid-phase matrix with said biological sample in order for said immobilized compound to form a complex by binding said Src-homology 2 domain containing protein or a phosphotyrosine binding domain containing protein
- removing unbound material from said biological sample and isolating said complex
- extracting said Src-homology 2 domain containing protein or a phosphotyrosine binding domain containing protein from said complex.

The present invention also relates to a compound of the invention coupled to a fluorescent or radioactive molecule.

The invention furthermore relates to a method for coupling a fluorescent or radioactive molecule to a compound of the invention comprising

- contacting said compound with said fluorescent or radioactive molecule in a reaction mixture to produce a complex
- removing uncomplexed material and isolating said complex from said reaction mixture.

The invention is further directed to a method for detecting protein-tyrosine phosphatase or other molecules with phosphotyrosine recognition unit(s) in a cell or in a subject using a compound of the invention coupled to a fluorescent or radioactive molecule comprising

- contacting said cell or an extract thereof or a biological sample from said subject or by injecting said compound into said subject in order for said compound to produce a complex with said protein-tyrosine phosphatase or said molecules with phosphotyrosine recognition unit(s)
- detecting said complex, thereby detecting the presence of said protein tyrosine phosphatase or said other molecules with phosphotyrosine recognition unit(s).

The invention is further directed to a method for quantifying the amount of protein-tyrosine phosphatases or other molecules with phosphotyrosine recognition unit(s) in a cell or in a subject using a compound of the invention coupled to a fluorescent or radioactive molecule comprising

- contacting said cell or an extract thereof or a biological sample from said subject or by injecting said compound into said subject in order for said compound to produce a

complex with said protein-tyrosine phosphatase or said molecules with phosphotyrosine recognition unit(s)

- measuring the amount of said complex, thereby detecting the presence of said protein tyrosine phosphatase or said molecules with phosphotyrosine recognition unit(s).

The invention is also concerned with a method for determining the function of a given protein-tyrosine phosphatase or group of protein-tyrosine phosphatases or said molecules with phosphotyrosine recognition unit(s) in a cell or a subject using a compound of the invention coupled to a fluorescent or radioactive molecule comprising

- contacting said cell or an extract thereof or a biological sample from said subject or by injecting said compound into said subject in order for said compound to produce a complex with said protein-tyrosine phosphatase or said molecules with phosphotyrosine recognition unit(s)
- measuring the biological effects induced by said complex.

Pharmacological Methods

For the above indications the dosage will vary depending on the compound of the invention employed, on the mode of administration and on the therapy desired. However, in general, satisfactory results are obtained with a dosage of from about 0.5 mg to about 1000 mg, preferably from about 1 mg to about 500 mg of compounds of the invention, conveniently given from 1 to 5 times daily, optionally in sustained release form. Usually, dosage forms suitable for oral administration comprise from about 0.5 mg to about 1000 mg, preferably from about 1 mg to about 500 mg of the compounds of the invention admixed with a pharmaceutical carrier or diluent.

The compounds of the invention may be administered in a pharmaceutically acceptable acid addition salt form or where possible as a metal or a C_{1-6}-alkylammonium salt. Such salt forms exhibit approximately the same order of activity as the free acid forms.

This invention also relates to pharmaceutical compositions comprising a compound of the invention or a pharmaceutically acceptable salt thereof and, usually, such compositions also contain a pharmaceutical carrier or diluent. The compositions containing the com-

pounds of this invention may be prepared by conventional techniques and appear in conventional forms, for example capsules, tablets, solutions or suspensions.

The pharmaceutical carrier employed may be a conventional solid or liquid carrier. Examples of solid carriers are lactose, terra alba, sucrose, talc, gelatine, agar, pectin, acacia, magnesium stearate and stearic acid. Examples of liquid carriers are syrup, peanut oil, olive oil and water.

Similarly, the carrier or diluent may include any time delay material known to the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

If a solid carrier for oral administration is used, the preparation can be tabletted, placed in a hard gelatine capsule in powder or pellet form or it can be in the form of a troche or lozenge. The amount of solid carrier will vary widely but will usually be from about 25 mg to about 1 g. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

Generally, the compounds of this invention are dispensed in unit dosage form comprising 10-200 mg of active ingredient in or together with a pharmaceutically acceptable carrier per unit dosage.

The dosage of the compounds according to this invention is 1-500 mg/day, e.g. about 100 mg per dose, when administered to patients, e.g. humans, as a drug.

A typical tablet that may be prepared by conventional tabletting techniques contains

Core:

Active compound (as free compound or salt thereof)	100 mg
Colloidal silicon dioxide (Areosil®)	1.5 mg
Cellulose, microcryst. (Avicel®)	70 mg
Modified cellulose gum (Ac-Di-Sol®)	7.5 mg
Magnesium stearate	

Coating:		
HPMC	approx.	9 mg
*Mywacett® 9-40 T	approx.	0.9 mg

*Acylated monoglyceride used as plasticiser for film coating.

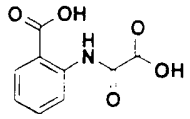
The route of administration may be any route which effectively transports the active compound to the appropriate or desired site of action, such as oral or parenteral e.g. rectal, transdermal, subcutaneous, intranasal, intramuscular, topical, intravenous, intraurethral, ophthalmic solution or an ointment, the oral route being preferred.

The process for preparing compounds of the invemtion is further illustrated in the following examples, which, however, are not to be construed as limiting.

EXAMPLES

Hereinafter, TLC is thin layer chromatography, $CDCl_3$ is deuterio chloroform, CD3OD is tetradeuterio methanol and DMSO-d_6 is hexadeuterio dimethylsulfoxide. The structures of the compounds are confirmed by either elemental analysis or NMR, where peaks assigned to characteristic protons in the title compounds are presented where appropriate. ^1H NMR shifts (δ_H) are given in parts per million (ppm) downfield from tetramethylsilane as internal reference standard. M.p.: is melting point and is given in °C and is not corrected. Column chromatography was carried out using the technique described by W.C. Still et al., J. Org. Chem. 43: 2923 (1978) on Merck silica gel 60 (Art. 9385). HPLC analyses are performed using 5µm C18 4 x 250 mm column eluted with various mixtures of water and acetonitrile, flow = 1 ml/min, as described in the experimental section.
Compounds used as starting material are either known compounds or compounds which can readily be prepared by methods known per se.
Wang-resin is polystyrene with a 4-hydroxymethylphenol ether linker.
2-Aminothiophenes are prepared according to Gewald *et al.*, *Chem. Ber. 99:* 94 (1966).
3-Aminothiophenes are prepared according to H. Hartmann and J. Liebscher, Synthesis 275 (1984).

EXAMPLE 1



2-(Oxalyl-amino)benzoic acid

To a stirred solution of anthranilic acid (20.1 g, 0.15 mol) in dry tetrahydrofuran (250 ml) was added dropwise ethyl oxalyl chloride (10.0 g, 0.073 mol). The resulting reaction mixture was stirred at room temperature for 15 min. filtered and the solvent evaporated in vacuo affording crude 16.4 g (94%) of 2-(ethoxyoxalyl-amino)benzoic acid as an oil.

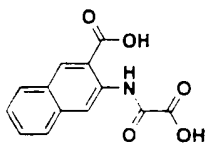
To a solution of the above benzoic acid (10.0 g, 0.042 mol) in ethanol (350 ml) was added a solution of sodium hydroxide (3.7 g, 0.092 mol) in water (100 ml). The resulting reaction mixture was stirred at room temperature for 60 h. Concentrated hydrochloric acid was added to pH = 1 and the precipitate was filtered off and washed with water (3 x 100 ml), diethyl ether (3 x 80 ml) and dried in vacuo affording 7.1 g (81%) of the title compound as a solid.

M.p.: 214 - 215 °C:

Calculated for $C_9H_7NO_5$, 0.2 H_2O;
C, 51.68 %, H, 3.37 %; N, 6.70 %. Found:
C, 50.96 %, H, 3.32 %; N, 6.52 %.

By a similar procedure as described in Example 1 the following compounds have been prepared.

EXAMPLE 2



3-(Oxalyl-amino)naphthalene-2-carboxylic acid:

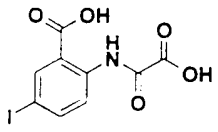
M.p.: 227 - 228 °C.

Calculated for $C_{13}H_9NO_5$:

C, 60.24 %; H, 3.50 %; N, 5.40 %. Found:

C, 59.98 %; H, 3.46 %; N, 5.25 %.

EXAMPLE 3



2-(Oxalyl-amino)-5-iodo-benzoic acid:

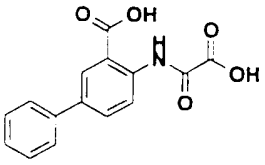
MS(ES): m/z = 326 (M+1)

Calculated for $C_9H_6NIO_5$, 0.75 x H_2O;

C, 31.01 %; H, 2.17 %; N, 4.02 %. Found:

C, 31.14 %; H, 2.33 %; N, 3.76 %.

EXAMPLE 4



4-(Oxalyl-amino)-biphenyl-3-carboxylic acid:

To a suspension of 5-bromo-2-amino-benzoic acid methyl ester (3.0 g, 13,04 mmol), tetrakis(triphenylphosphine)palladium(0) (0.5 g, 0.44 mmol), toluene (40 ml) and 2N aqueous sodium carbonate (14.8 ml) was added a solution of phenylboronic acid (2.2 g, 17.73 mmol) in methanol (10 ml) at room temperature. The resulting reaction mixture was heated at reflux temperature for 4 h. cooled and diluted with water (50 ml). The insoluble matter was filtered off and the phases were separated. The aqueous phase was extracted with ethyl acetate

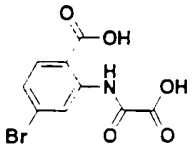
(100 ml) and the combined organic phases were washed with water (2 x 80 ml), diluted aqueous ammoniac (80 ml) and saturated aqueous sodium chloride (80 ml). The organic phase was dried ($MgSO_4$), filtered and evaporated in vacuo affording 3.4 g of crude 4-amino-biphenyl-3-carboxylic acid methyl ester which was purified on silicagel (1 l) using a mixture of ethyl acetate and heptane (1:3) as eluent. Pure fractions were collected and evaporated in vacuo affording 2.7 g (91 %) of 4-amino-biphenyl-3-carboxylic acid methyl ester.

4-Amino-biphenyl-3-carboxylic acid methyl ester was converted into the title compound by a similar procedure as described in Example 1.

M.p.: 223 - 224 °C.

Calculated for $C_{15}H_{11}NO_5$, 0.5 x H_2O;
C, 61.23 %; H, 4.11 %; N, 4.76 %. Found:
C, 60.96 %; H, 4.01 %; N, 4.62 %.

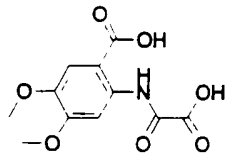
EXAMPLE 5



4-Bromo-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (d, J = 7.5 Hz, 1H), 8.25 (s, 1H), 7.80 (d, J = 7.5 Hz, 1H).
MS: ESI (-): 288 [M-1(^{81}Br)), 287 (M-1(^{80}Br)].

EXAMPLE 6

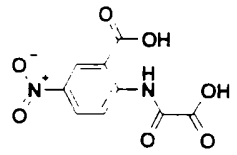


4,5-Dimethoxy-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (s, 1H), 7.60 (s, 1H), 3.95 (s, 3H), 3.86 (s, 3H).

MS: ESI (-): 268 [M-1].

EXAMPLE 7

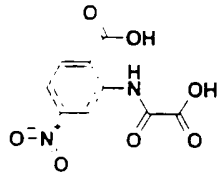


5-Nitro-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 8.90 (d, J = 7.5Hz, 2H), 8.42 (s, 1H).

MS: ESI (-): 253 [M-1].

EXAMPLE 8

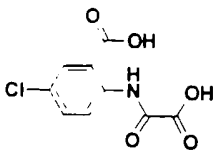


4-Nitro-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 9.60 (s, 1H), 8.36 (m, 1H), 8.02 (m, 1H).

MS: ESI (-): 253 [M-1].

EXAMPLE 9

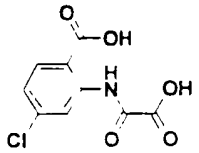


5-Chloro-2-(oxalyl-amino)-benzoic acid:

^{1}H NMR (400 MHz, CD_3OD) δ 8.72 (d, J = 7.5 Hz, 1H), 8.10 (s, 1H), 7.60 (d, J = 7.5 Hz, 1H).

MS: ESI (-): 242 [M-1(^{35}Cl)], 244 [M-1(^{37}Cl)].

EXAMPLE 10

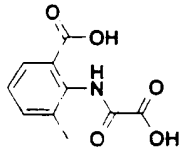


4-Chloro-2-(oxalyl-amino)-benzoic acid:

^{1}H NMR (400 MHz, CD_3OD) δ 8.80 (s, 1H), 8.10 (d, J = 7.5 Hz, 1H), 7.22 (d, J = 7.5 Hz, 1H).

MS: ESI (-): 242 [M-1(^{35}Cl)], 244 [M-1(^{37}Cl)].

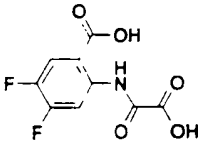
EXAMPLE 11



3-Methyl-2-(oxalyl-amino)-benzoic acid:

^{1}H NMR(400 MHz, DMSO-d_6) δ 2.2 (s, 3H), 7.2 - 7.7 (m, 3H), 10.5 (s, 1H), 12.9 (s, 1H).

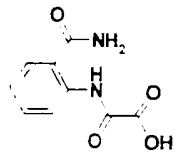
EXAMPLE 12



4,5-Difluoro-2-(oxalyl-amino)-benzoic acid:

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.03 (m, 1H), 8.61 (dd, 1H), 12.55 (s, 1H, N*H*CO).

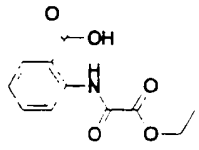
EXAMPLE 13



N-(2-Carbamoyl-phenyl)-oxalamic acid;

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.20 (t, 1H), 7.55 (t, 1H), 7.73 (bs, 1H, CON*H*$_2$), 7.83 (d, 1H), 8.30 (bs, 1H, CON*H*$_2$), 8.52 (d, 1H), 12.9 (s, 1H, N*H*CO).

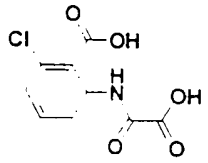
EXAMPLE 14



2-(Ethoxyoxalyl-amino)-benzoic acid;

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (t, 3H), 4.30 (q, 2H), 7.24 (t, 1H), 7.65 (t, 1H), 8.03 (d, 1H), 8.56 (d, 1H), 12.6 (s, 1H, N*H*CO).

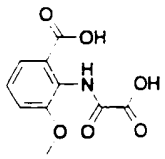
EXAMPLE 15



6-Chloro-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.68 (bs, 1H), 8.06 (d, J = 9 Hz, 1H), 7.43 (t, J = 9 Hz, 1H), 7.27 (d, J = 9 Hz, 1H).

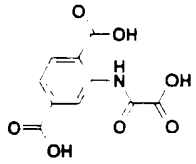
EXAMPLE 16



3-Methoxy-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (bs, 1H), 7.37 - 7.25 (m, 3H), 3.80 (s, 3H).

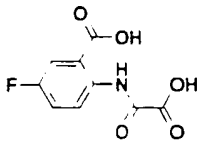
EXAMPLE 17



2-(Oxalyl-amino)-terephthalic acid;

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28 (s, 1H), 8.22 (d, J = 9 Hz, 1H), 7.75 (d, J = 9 Hz, 1H).

EXAMPLE 18

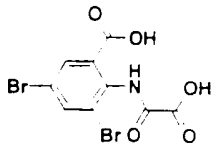


5-Fluoro-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 7.50 (m, 1H), 7.25 (m, 1H), 7.22 (m, 1H).

MS m/z 227.2 (M-1).

EXAMPLE 19

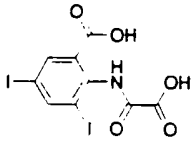


3,5-Dibromo-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 8.08 (s, 1H), 8.05 (s, 1H).

MS m/z 366.1 (M-1).

EXAMPLE 20

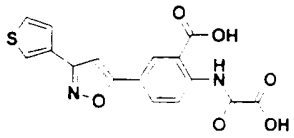


3,5-Diiodo-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 8.45 (s, 1H), 8.25 (s, 1H).

MS m/z 460.1 (M-1).

EXAMPLE 21



2-(Oxalyl-amino)-5-(3-thiophen-3-yl-isoxazol-5-yl)-benzoic acid;

To a solution of thiophene-3-carboxaldehyde (2.0 g, 18 mmol) in 1,4-dioxane (6.0 ml) was added hydroxylamine hydrochloride (1.24 g, 18 mmol) and triethylamine (2.5 ml, 18 mmol). The mixture was sonicated for 0.5 h and stirred at room temperature for 116 h and at 35 °C for 48 hour. The solvent was removed in vacuo and the residue was dissolved in di-

chloromethane and washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo affording 1.97 g (87 %) of thiophene-3-carbaldehyde oxime as an oil.

To a solution of the above thiophene-3-carbaldehyde oxime (120 mg, 0.99 mmol) and 2-(*tert*-butoxyoxalyl-amino)-5-ethynyl-benzoic acid methyl ester (100 mg, 0.33 mmol) in tetrahydrofuran (2.5 ml) stirred at room temperature was added 0.75 M bleach (1.3 ml, 0.99 mmol). The solution was first stirred at room temperature for 24 h and then at 35 °C for 24 h. The solvent was evaporated in vacuo and the residue was dissolved in dichloromethane, washed with water, brine and dried ($MgSO_4$), filtered and evaporated in vacuo. The residual film was purified by preparative TLC affording 21 mg, (15 %) of 2-(*tert*-butoxyoxalyl-amino)-5-(3-thiophen-3-yl-isoxazol-5-yl)-benzoic acid methyl ester as an oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.75 (s, 1H), 8.92 (d, 1H, J = 11 Hz), 8.59 (s, 1H), 8.06 (d, 1H, J = 11 Hz), 7.91 (s, 1H), 7.59 (d, 1H, J = 7 Hz), 7.28 (d, 1H, J = 7 Hz), 6.90 (s, 1H), 4.07 (s 3H), 1.65 (s, 9H).

The above 2-(*tert*-butoxyoxalyl-amino)-5-(3-thiophen-3-yl-isoxazol-5-yl)-benzoic acid methyl ester (10 mg, 0.023 mmol) was dissolved in 20 % trifluoroacetic acid/dichloromethane (0.3 ml) and stirred at room temperature for 21 h. The solvent was removed in vacuo affording 8.4 mg (98 %) of 2-(oxalyl-amino)-5-(3-thiophen-3-yl-isoxazol-5-yl)-benzoic acid methyl ester as a solid.

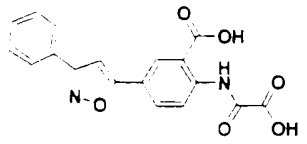
^{1}H NMR (400 MHz, CD_3OD) δ 12.75 (s, 1H), 8.95 (d, 1H, J = 11 Hz), 8.62 (s, 1H), 8.18 (d, 1H, J = 11 Hz), 7.75 (m, 1H), 7.65 (m, 1H), 7.60 (s, 1H), 4.07 (s, 3H).

To a solution of 2-(oxalyl-amino)-5-(3-thiophen-3-yl-isoxazol-5-yl)-benzoic acid methyl ester (8.4 mg, 0.023 mmol) in methanol (1.5 ml) and tetrahydrofuran (0.5 ml) at room temperature was added 1 N lithium hydroxide (90 µl, 0.090 mmol). The solution was stirred for 48 h. The solvent was removed in vacuo and the residue was redissolved in water. The solution was acidified with 1 N hydrochloric acid to pH = 1 and extracted with ethyl acetate. The combined extracts were washed with brine, dried ($MgSO_4$), filtered and the solvent evaporated in vacuo affording 6.4 mg (79 %) of the title compound as a solid.

^{1}H NMR (400 MHz, CD_3OD) δ 8.75 (d, 1H, J=11 Hz), 8.70 (s, 1H), 7.95 (s, 1H), 7.82 (d, 1H, J = 11 Hz), 7.70 (m, 1H), 7.60 (m, 1H).

MS m/z: 357(M-1).

EXAMPLE 22



2-(Oxalyl-amino)-5-(3-phenyl-isoxazol-5-yl)-benzoic acid:

To a solution of benzaldehyde (2.0 g, 19 mmol) in 1,4-dioxane (6.0 ml) was added hydroxylamine hydrochloride (1.3 g, 19 mmol) and triethylamine (2.6 ml, 19 mmol). The mixture was sonicated for 0.5 h and stirred at room temperature for 116 h and at 35 °C for 24 h. The solvent was removed in vacuo and the residue was dissolved in dichloromethane and washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo affording 1.9 g (84 %) of benzaldehyde oxime as an oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.60 (m, 2H), 7.41 (m, 3H).

To a solution of benzaldehyde oxime (120 mg, 0.99 mmol) and 2-(*tert*-butoxyoxalyl-amino)-5-ethynyl-benzoic acid methyl ester (100 mg, 0.33 mmol) in tetrahydrofuran (2.5 ml) stirred at room temperature was added 0.75 M bleach (1.3 ml, 0.99 mmol). The solution was first stirred at room temperature for 24 h and then at 35 °C for 24 h. The solvent was evaporated in vacuo and the residue was dissolved in dichloromethane. The solution was washed with water, brine, dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residue was washed with diethyl ether to get a solid precipitate which was filtered off yielding 59 mg (42 %) of 2-(*tert*-butoxyoxalyl-amino)-5-(3-phenyl-isoxazol-5-yl)-benzoic acid methyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.75 (s, 1H), 8.85 (d, 1H, J = 11 Hz), 8.62 (s, 1H), 8.06 (d, 1H, J = 11 Hz), 7.91 (m, 2H), 7.52 (m, 3H), 6.90 (s, 1H), 4.07 (s 3H), 1.65 (s, 9H).

The above 2-(*tert*-butoxyoxalyl-amino)-5-(3-phenyl-isoxazol-5-yl)-benzoic acid methyl ester (28 mg, 0.07 mmol) was dissolved in 20 % trifluoroacetic acid/dichloromethane

(0.5 ml) and stirred at room temperature for 6 h. The solvent was removed in vacuo affording 25 mg (100 %) of 2-amino-5-(3-phenyl-isoxazol-5-yl)-benzoic acid methyl ester as a solid.

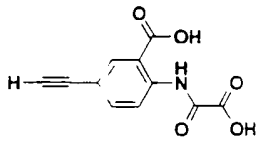
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.75 (s, 1H), 8.85 (d, 1H, J = 11 Hz), 8.62 (s, 1H), 8.15 (d, 1H, J = 11 Hz), 7.91 (m, 2H), 7.52 (m, 3H), 6.90 (s, 1H), 4.07 (s 3H)

To a solution of the above 2-amino-5-(3-phenyl-isoxazol-5-yl)-benzoic acid methyl ester (12.5 mg, 0.034 mmol) in methanol (2.5 ml) and tetrahydrofuran (1.0 ml) at room temperature was added 1 N lithium hydroxide (1.4 ml, 0.136 mmol). The solution was stirred for 12 h and the solvent was removed in vacuo. The residue was dissolved in water acidified with 1 N hydrochloric acid to pH = 1 and extracted with ethyl acetate. The organic extract was washed with brine, dried ($MgSO_4$), filtered and the solvent evaporated in vacuo affording 7.7 mg (64 %) of the title compound as a solid.

^{1}H NMR (400 MHz, CD_3OD) δ 8.91 (d, 1H, J = 11 Hz), 8.62 (s, 1H), 8.15 (d, 1H, J = 11 Hz), 7.91 (m, 2H), 7.49 (m, 3H), 7.25 (s, 1H), 4.07 (s 3H).

LC/MS m/z: 351(M-1).

EXAMPLE 23



5-Ethynyl-2-(oxalyl-amino)-benzoic acid:

A solution of 2-(*tert*-butoxyoxalyl-amino)-5-iodo-benzoic acid (5.0 g, 12.8 mmol) and N,N-dimethylformamide di-tertbutylacetal (12 ml, 51.2 mmol) in toluene (100 ml) was heated at reflux for 20 h. The reaction was cooled to room temperature, concentrated in vacuo and the residue dissolved in ethyl acetate (150 ml). The ethyl acetate phase was washed with water (3 x 35 ml), brine (20 ml) and the volatiles evaporated in vacuo. The residue was purified by silica gel chromatography using 25 % ethyl acetate/hexane as eluent. Pure fractions were combined and concentrated in vacuo to yield 2.3 g of 2-(*tert*-butoxyoxalyl-amino)-5-iodo-benzoic acid tert-butyl ester as an oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, J = 9 Hz, 1H), 8.27 (s, 1H), 7.83 (d, J = 9 Hz, 1H), 1.62 (s, 18H).

2-(*tert*-Butoxyoxalyl-amino)-5-iodo-benzoic acid tert-butyl ester (0.83 g, 1.86 mmol), trimethylsilyl acetylene (2 ml), and triethylamine (1 ml, 7.44 mmol) were dissolved in N,N-dimethylformamide (5 ml) and the solution purged with argon. Dichlorobis-(triphenylphosphine)palladium(II) (26 mg, 0.15 mmol) and copper(I)iodide (4 mg, 0.15 mmol) were added and the reaction stirred at 60 °C under argon for 5 h. The crude mixture was diluted with ethyl acetate (40 ml) and washed with water (3 x 10 ml) and brine (2 x 10 ml). The solvent was evaporated in vacuo to yield 0.77 g (99 %) of 2-(*tert*-butoxyoxalyl-amino)-5-trimethylsilanylethyny-benzoic acid *tert*-butyl ester.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.59 (s, 1H), 8.71 (d, J = 9 Hz, 1H), 8.07 (d, J = 9 Hz, 1H), 1.62 (s, 9H), 1.61 (s, 9H), 0.25 (s, 9H).

2-(*tert*-Butoxyoxalyl-amino)-5-trimethylsilanylethyny-benzoic acid *tert*-butyl ester (0.57 g, 1.37 mmol) was dissolved in tetrahydrofuran (5 ml) and treated with a 0.9 M solution of tetrabutylammonium fluoride and acetic acid (2:3) in tetrahydrofuran (1.7 ml, 1.51 mmol) for 3 h. The volatiles were evaporated in vacuo and the crude material extracted into ethyl acetate (35 ml). The ethyl acetate extract was washed with 1M hydrochloric acid (5 ml), saturated sodium bicarbonate (5 ml), brine (5 ml) and evaporated in vacuo affording 0.36 g (76 %) of 2-(*tert*-butoxyoxalyl-amino)-5-ethynyl-benzoic acid *tert*-butyl ester as an oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, J = 10 Hz, 1H), 8.12 (s, 1H), 7.65 (d, J = 10 Hz, 1H), 3.08 (s, 1H), 1.62 (s, 9H), 1.58 (s, 9H).

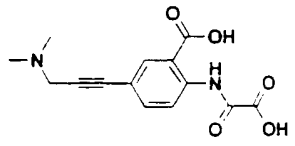
2-(*tert*-Butoxyoxalyl-amino)-5-ethynyl-benzoic acid *tert*-butyl ester (0.36 g, 1.04 mmol) was treated with 50 % trifluoroacetic acid/dichloromethane (15 ml) at room temperature for 3 h. The reaction mixture was concentrated in vacuo and the residue was washed with water and diethyl ether affording after drying 0.21 g (86 %) of the title compound.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 10 Hz, 1H), 8.05 (s, 1H), 7.76 (d, J = 10 Hz, 1H), 4.24 (s, 1H).

LC/MS $[M-H]^-$: 232.07

HPLC (254.4 nm): 3.112 s. (49 %).

EXAMPLE 24



5-(3-Dimethylamino-prop-1-ynyl)-2-(oxalyl-amino)-benzoic acid:

To a solution of 5-Iodoanthranilic acid (3.0 g, 11.4 mmol) and N,N-diisopropylethylamine (4 ml, 22.8 mmol) in anhydrous tetrahydrofuran (40 ml) was added imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (4.47g, 22.8 mmol). The reaction was stirred at room temperature for 3 h. The solvents were evaporated in vacuo and the crude mixture extracted into ethyl acetate (70 ml). The organic extract was washed with 1% hydrochloric acid (2 x 15 ml) and brine (10 ml) and the solvent was evaporated in vacuo affording 2.8 g (63 %) of 2-(*tert*-butoxyoxalyl-amino)-5-iodo-benzoic acid as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, J = 9 Hz, 1H), 8.43 (d, J = 2 Hz, 1H), 8.00 (dd, J = 9 Hz, 2 Hz, 1H), 1.59 (s, 9H).

To a solution of 2-(*tert*-butoxyoxalyl-amino)-5-iodo-benzoic acid (2.1 g, 5.37 mmol) in dichloromethane (15 ml) under nitrogen was added triethylamine (3.75 ml, 26.85 mmol) and N,N-dimethylaminopyridine (0.1 g). Methoxymethyl chloride (1.2 ml, 16.11 mmol) was added and the reaction mixture was stirred for 4 h and concentrated in vacuo to a minimum volume which was loaded directly onto a silica gel column, eluting with 50 % ethyl acetate/hexane. Pure fractions were combined and concentrated to give 1.5 g (64 %) of 2-(*tert*-butoxyoxalyl-amino)-5-iodo-benzoic acid methoxymethyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, J = 9 Hz, 1H), 8.42 (s, 1H), 7.89 (d, J = 9 Hz, 1H), 5.54 (s, 2H), 3.60 (s, 3H), 1.61 (s, 9H).

A solution of 2-(*tert*-butoxyoxalyl-amino)-5-iodo-benzoic acid methoxymethyl ester (0.16 g, 0.37 mmol), triethylamine (51 µl, 0.37 mmol) and 1-dimethylamino-2-propyne (0.12 ml, 1.11 mmol) was prepared in anhydrous acetonitrile (3 ml) and purged with argon. Dichlorobis-

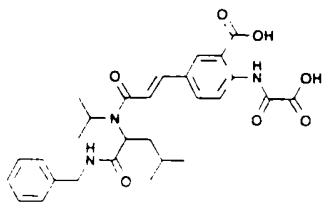
(triphenylphosphine)palladium (II) (5 mg, 0.0074 mmol) and copper(I) iodide (1 mg, 0.0074 mmol) were added and the reaction stirred at 60 °C under argon for 18 h. The volatiles were evaporated in vacuo and the residue redissolved in ethyl acetate (10 ml). The organic phase was washed with 1% hydrochloric acid (5 ml) and the aqueous phase extracted with additional ethyl acetate. The combined organic extracts were washed with brine (5 ml), dried (Na_2SO_4) and concentrated to an oil. The crude oil was dissolved in dichloromethane and purified by silica gel chromatography using 5 % methanol/dichloromethane/0.1 % triethylamine as eluent. Pure fractions were combined to give 81 mg (60 %) of 2-(*tert*-butoxyoxalyl-amino)-5-(3-dimethylamino-prop-1-ynyl)-benzoic acid methoxymethyl ester as an oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.52 (s, 1H), 8.75 (d, J = 9 Hz, 1H), 8.21 (s, 1H), 7.64 (d, J = 9 Hz, 1H), 5.53 (s, 2H), 3.59 (s, 3H), 3.46 (s, 2H), 2.38 (s, 6H), 1.61 (s, 9H).

2-(*tert*-Butoxyoxalyl-amino)-5-(3-dimethylamino-prop-1-ynyl)-benzoic acid methoxymethyl ester (32.3 mg) was treated with 50 % trifluoroacetic acid/dichloromethane (3 ml) at room temperature for 2 h. The mixture was concentrated to a solid in vacuo and the resulting solid was washed with dichloromethane which afforded 20 mg (83 %) of the title compound as a solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 9 Hz, 1H), 8.05 (s, 1H), 7.60 (d, J = 9 Hz, 1H), 4.07 (s, 2H), 2.73 (s, 6H).

EXAMPLE 25



5-(2-((1-Benzylcarbamoyl-3-methyl-butyl)-isopropyl-carbamoyl)-vinyl)-2-(oxalyl-amino)-benzoic acid:

5-(2-((1-Benzylcarbamoyl-3-methyl-butyl)-isopropyl-carbamoyl)-vinyl)-2-(*tert*-butoxyoxalyl-amino)-benzoic acid methoxymethyl ester (0.14 g, 0.22 mmol) was treated with a solution of

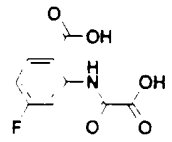
50 % trifluoroacetic acid/dichloromethane (6 ml) for 2.5 h. The mixture was concentrated in vaçuo and precipitated from water. The resulting crystalline solid was filtered off and dried in vaçuo to give 0.10 g (85 %) of the title compound as a solid.

^{1}H NMR (400 MHz, CD_3OD) δ 8.76 (d, J = 9 Hz, 1H), 8.31 (s, 1H), 7.94 (d, J = 9 Hz, 1H), 7.49 (d, J = 16 Hz, 1H), 7.46 - 7.38 (m, 5H), 7.13 (d, J = 16 Hz, 1H), 4.49 (m, 1H), 4.10 (s, 2H), 3.95 (m, 1H), 2.49 (m, 1H), 1.90 (m, 1H), 1.56 (m, 1H), 1.38 (d, J = 6 Hz, 3H), 1.35 (d, J = 7 Hz, 3H), 0.97 (d, J = 6 Hz, 6H).

LC/MS $[M-H]^-$: 522.55.

The following compounds were prepared in a similar way as described in Example 1.

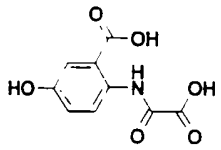
EXAMPLE 26



4-Fluoro-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.11 (m, 1H), 8.12 (m, 1H), 8.42 (dd, 1H), 12.62 (s, 1H, N*H*CO).

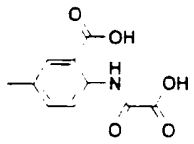
EXAMPLE 27



5-Hydroxy-2-(oxalyl-amino)-benzoic acid;

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H) 9.78 (bs, 1H) 8.44 (d, J = 10 Hz, 1H), 7.42 (d, J = 2 Hz, 1H), 7.05 (dd, J = 10 Hz, 2 Hz).

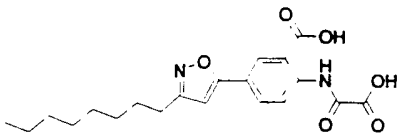
EXAMPLE 28



5-Methyl-2-(oxalyl-amino)-benzoic acid:

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H), 8.51 (d, J = 10 Hz, 1H), 7.83 (d, J = 2 Hz, 1H), 7 42 (dd, J = 10 Hz, 2 Hz, 1H), 2.29 (s, 3H).

EXAMPLE 29



5-(3-Octyl-isoxazol-5-yl)-2-(oxalyl-amino)-benzoic acid:

To a solution of 2-(*tert*-butoxyoxalyl-amino)-5-iodo-benzoic acid (1.8 g, 4.6 mmol) and potassium carbonate (1.6 g, 11.5 mmol) in acetone (15 ml) was added iodomethane (3 g). The reaction was heated at reflux for 2 h. after which it was judged complete by tlc analysis. The crude mixture was diluted with ethyl acetate (75 ml), washed with water (2 x 15 ml), and brine (10 ml). The organic phase was concentrated in vacuo to give 1.8 g (94 %) of 2-(*tert*-butoxyoxalyl-amino)-5-iodo-benzoic acid methyl ester.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.52 (s, 1H), 8.53 (d, J = 9 Hz, 1H), 8.39 (s, 1H), 8.87 (d, J = 9 Hz, 1H), 3.98 (s, 3H), 1.61 (s, 9H).

2-(*tert*-Butoxyoxalyl-amino)-5-iodo-benzoic acid methyl ester (0.86 g, 2.12 mmol), trimethylsilyl acetylene (2 ml), and triethylamine (1.2 ml, 8.48 mmol) were dissolved in N,N-dimethylformamide (5 ml) and the solution purged with argon. Dichlorobis-

(triphenylphosphine)palladium (II) (30 mg, 0.042 mmol) and copper(I)iodide (4 mg, 0.021 mmol) were added and the reaction stirred at 60 °C under argon for 3 h. The crude mixture was diluted with ethyl acetate (40 ml) and washed with water (3 x 10 ml) and brine (2 x 10 ml). The organic phase was evaporated in vacuo to yield 0.8 g (99 %) of 2-(*tert*-Butoxyoxalyl-amino)-5-trimethylsilanylethyny-benzoic acid methyl ester which was used without further purification.

2-(*tert*-Butoxyoxalyl-amino)-5-trimethylsilanylethyny-benzoic acid methyl ester (0.15 g, 0.4 mmol) was dissolved in tetrahydrofuran (2 ml) and treated with a 0.9 M solution of tetrabutylammonium fluoride and acetic acid (2:3) in tetrahydrofuran (0.44 ml, 0.4 mmol) for 3 h. The volatiles were evaporated in vacuo and the crude material extracted into ethyl acetate (25 ml). The ethyl acetate extract was washed with 1M hydrochloric acid (5 ml), saturated sodium bicarbonate (5 ml), brine (5 ml) and evaporated in vacuo affording 0.1 g (83 %) of 2-(*tert*-butoxyoxalyl-amino)-5-ethynyl-benzoic acid methyl ester as an oil.

A solution of 2-(*tert*-butoxyoxalyl-amino)-5-ethynyl-benzoic acid methyl ester (0.1 g, 0.33 mmol) and nonaldoxime (0.15 g, 0.99 mmol) in tetrahydrofuran (3 ml) was treated with bleach (0.75 M, 1.3 ml, 0.99 mmol). The reaction was stirred at room temperature for 24 h. Tlc analysis showed presence of starting material so the reaction was heated to 35 °C for 12 h. The solvents were removed in vacuo and the crude material was dissolved in ethyl acetate (35 ml), washed with water (2 x 10 ml) and brine (10 ml). The organic extract was evaporated in vacuo affording 0.1 g (66 %) of 2-(*tert*-butoxyoxalyl-amino)-5-(3-octyl-isoxazol-4-yl)-benzoic acid methyl ester as an oil.

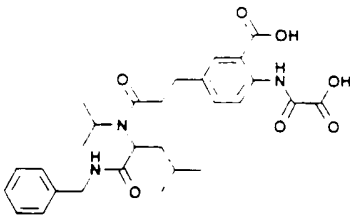
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (d, J = 10 Hz, 1H), 8.52 (d, J = 1 Hz, 1H), 7.97 (dd, J = 10, 1 Hz, 1H), 6.41 (s, 1H), 4.02 (s, 3H), 2.73 (t, J = 8 Hz, 2H), 1.70 (m, 2H), 1.62 (s, 9H), 1.37 - 1.25 (bm, 12H), 0.89 (t, J = 8 Hz, 3H).

To a solution of the above (isoxazol-4-yl)-benzoic acid methyl ester (9.1 mg, 0.02 mmol) in 50 % methanol/tetrahydrofuran (2 ml) was added 1N lithium hydroxide (60 µl, 0.06 mmol) and the resulting mixture was stirred at room temperature for 48 h. The reaction was judged to be incomplete by tlc analysis (30 % methanol/dichloromethane) and additional 1 N lithium hydroxide was added (20 µl, 0.02 mmol). The reaction was stirred for another 72 hours. pH of the reaction mixture was adjusted to around 0 by addition of 1 N hydrochloric acid. The mixture was concentrated in vacuo and the crude material was dissolved in ethyl acetate (20

ml). The organic layer was washed with brine (2 x 5 ml) and concentrated in vacuo affording 5.4 mg (70 %) of the title compound as a soild.

^{1}H NMR (400 MHz, CD_3OD) δ 8.87 (d, J = 10 Hz, 1H), 8.55 (s, 1H), 8.04 (d, J = 10 Hz, 1H), 6.74 (s, 1H), 2.70 (t, J = 8 Hz, 2H), 1.72 (m, 2H), 1.38 -1.20 (bm, 12H), 0.90 (t, J = 8 Hz, 3H).

EXAMPLE 30



5-(2-((1-Benzylcarbamoyl-3-methyl-butyl)-isopropyl-carbamoyl)-ethyl)-2-(oxalyl-amino)-benzoic acid:

To a solution of isopropylamine (0.43 ml, 5.0 mmol) in methanol (5 ml) was added isovaleraldehyde (0.54 ml, 5.0 mmol). After 15 minutes of stirring a solution of benzylisocyanide in tetrahydrofuran (1 M, 5 ml, 5.0 mmol) was added followed by acrylic acid (0.34 ml, 5.0 mmol). The reaction was stirred at room temperature for 72 h, the volatiles were removed in vacuo and the resulting oil dissolved in ethyl acetate (40 ml). The organic mixture was washed with 1N hydrochloric acid (10 ml) and brine (10 ml), dried (Na_2SO_4) and the solvent evaporated in vacuo. The crude residue was purified by chromatography using a gradient from 30 % ethyl acetate/hexane to 50 % ethyl acetate/hexane. Pure fraction were collected and the solvent evaporated in vacuo which afforded 1.5 g (100 %) of N-(1-benzylcarbamoyl-3-methyl-butyl)-N-isopropyl-acrylamide as an oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (bs, 1H), 7.30 - 7.19 (m, 5H), 6.49 (dd, J = 16 Hz, 12 Hz, 1H), 6.25 (d, J = 16 Hz, 1H), 5.66 (d, J = 12 Hz, 1H), 4.38 (d, J = 6 Hz, 2H), 4.10 - 4.02 (m, 1H), 2.22 - 2.13 (m, 1H), 1.76 - 1.70 (m, 1H), 1.62 - 1.54 (m, 2H), 1.25 (d, J = 7 Hz, 3H), 1.20 (d, J = 7 Hz, 3H), 0.94 (d, J = 7 Hz, 3H), 0.90 (d, J = 7 Hz, 3H).

A solution of N-(1-benzylcarbamoyl-3-methyl-butyl)-N-isopropyl-acrylamide (0.55 g, 1.74 mmol), 2-(*tert*-butoxyoxalyl-amino)-5-iodo-benzoic acid methoxymethyl ester (0.5 g, 1.15 mmol), palladium acetate (3.0 mg, 0.023 mmol) and tri(o-tolyl)phosphine (10.0 mg, 0.07 mmol) in N,N-dimethylformamide under argon and heated to 100 °C with stirring for 3 h. The reaction was cooled to room temperature and diluted in ethyl acetate (50 ml). The organic phase was washed with water (2 x 15 ml) and brine (10 ml), dried (Na_2SO_4) and evaporated in vacuo. The crude oily material was purified by chromatography using 30 % ethyl acetate/hexane as eluent. Pure fractions were collects and the solvent evaporated in vacuo affording 0.15 g (20 %) of 5-(2-((1-Benzylcarbamoyl-3-methyl-butyl)-isopropyl-carbamoyl)-vinyl)-2-(*tert*-butoxyoxalyl-amino)-benzoic acid methoxymethyl ester as an oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.58 (s, 1H), 8.82 (d, J = 9 Hz, 1H), 8.22 (s, 1H), 7.80 (d, J = 9 Hz, 1H), 7.60 (d, J = 16 Hz, 1H), 7.30 - 7.22 (m, 5H), 6.80 (d, J = 16 Hz, 1H), 5.58 (s, 2H), 4.43 (bs, 2H), 4.21 - 4.15 (m, 1H), 3.60 (s, 3H), 2.21 -2.16 (m, 1H), 1.82 - 1.78 (m, 1H), 1.61 (s, 9H), 1.61 - 1.58 (m, 1H), 1.35 (d, J = 7 Hz, 3H), 1.24 (t, J = 7 Hz, 3H), 0.99 (d, J = 7 Hz, 3H), 0.94 (d, J = 7 Hz, 3H).

To a solution of 5-(2-((1-benzylcarbamoyl-3-methyl-butyl)-isopropyl-carbamoyl)-vinyl)-2-(*tert*-butoxyoxalyl-amino)-benzoic acid methoxymethyl ester (10.7 mg, 0.017 mmol) in methanol (1 ml) was added 5 % palladium/carbon (2.2 mg) and the resulting mixture was stirred under hydrogen gas (30 psi) for 3 h. The mixture was filtered through celite and evaporated in vacuo. NMR indicated that the reaction was not complete so it was subjected to the hydrogenation conditions for another 4 h. The mixture was filtered and evaporated in vacuo again affording 8.9 mg (83 %) of 5-(2-((1-benzylcarbamoyl-3-methyl-butyl)-isopropyl-carbamoyl)-ethyl)-2-(tert-butoxyoxalyl-amino)-benzoic acid methoxymethyl ester as an oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.41 (s, 1H), 8.68 (d, J = 9 Hz, 1H), 8.07 (bs, 1H), 7.98 (s, 1H), 7.43 (d, J = 9 Hz, 1H), 7.30 - 7.22 (m, 5H), 5.52 (s, 2H), 4.45 - 4.33 (m, 2H), 4.04 - 3.96 (m, 2H), 3.58 (s, 3H), 2.95 (t, J = 7 Hz, 2H), 2.72 - 2.61 (m, 2H), 2.30 (m, 1H), 1.62 (s, 9H), 1.59 (m, 1H partially obscured by neighboring singlet), 1.22 (d, J = 6 Hz, 6H), 0.95 (d, J = 7 Hz, 3H), 0.91 (d, J = 7 Hz, 3H).

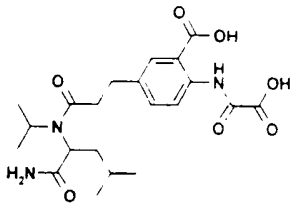
5-(2-((1-Benzylcarbamoyl-3-methyl-butyl)-isopropyl-carbamoyl)-ethyl)-2-(tert-butoxyoxalyl-amino)-benzoic acid methoxymethyl ester (4 mg, 0.0064 mmol) was dissolved in acetone (3 ml) and treated with 3 drops of 1N hydrochloric acid. The reaction was stirred for 2 days, after which the acetone was evaporated in vacuo. The residue was dissolved in ethyl acetate

(10 ml), washed with brine (2 x 2ml) and evaporated in vacuo. The resulting oil was treated with 20 % trifluoroacetic acid/dichloromethane (3 ml) for 3 h. The volatiles were evaporated in vacuo affording 2 mg (61 %) of the title compound as an oil.

^{1}H NMR (400 MHz, CD_3OD) δ 8.64 (d, J = 9 Hz, 1H), 8.00 (s, 1H), 7.51 (d, J = 9 Hz, 1H), 7.50 - 7.40 (m, 5H), 4.17 (t, J = 8 Hz, 1H), 4.14 (s, 2H), 3.73 (m, 1H), 2.95 (t, J = 6 Hz, 2H), 2.82 - 2.63 (m, 2H), 2.42 (m, 1H), 1.80 (m, 1H), 1.30 (m, 1H), 1.26 (d, J = 6 Hz, 3H), 1.10 (d, J = 6 Hz, 3H), 0.90 (d, J = 6 Hz, 6H).

LC/MS $[M-H]^-$: 524.74

EXAMPLE 31



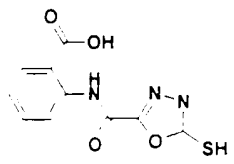
5-(2-((1-Carbamoyl-3-methyl-butyl)-isopropyl-carbamoyl)-ethyl)-2-(oxalyl-amino)-benzoic acid;

5-(2-((1-Benzylcarbamoyl-3-methyl-butyl)-isopropyl-carbamoyl)-vinyl)-2-(oxalyl-amino)-benzoic acid (33 mg, 0.063 mmol) and 10 % palladium/carbon was mixed in methanol (3 ml) and stirred under hydrogen gas (1 atm) for 18 h. The mixture was filtered through celite and the volatiles were evaporated in vacuo affording 27 mg (99 %) of the title compound.

^{1}H NMR (400 MHz, CD_3OD) δ 8.64 (d, J = 9 Hz, 1H), 8.00 (s, 1H), 7.51 (d, J = 9 Hz, 1H), 4.17 (t, J = 8 Hz, 1H), 3.72 (m, 1H), 2.96 (t, J = 6 Hz, 2H), 2.82-2.63 (m, 2H), 2.41 (m, 1H), 1.80 (m, 1H), 1.30 (m, 1H), 1.25 (d, J = 6 Hz, 3H), 1.13 (d, J = 6 Hz, 3H), 0.90 (d, J = 6 Hz, 6H).

LC/MS $[M-H]^-$: 435.66

EXAMPLE 32



2-((5-Mercapto-[1,3,4]oxadiazole-2-carbonyl)-amino)-benzoic acid:

To a solution of 2-(Ethoxyoxalyl-amino)-benzoic acid (2.0 g, 8.43 mmol) in ethanol (75 ml) was added hydrazine hydrate (0.8 g, 16.86 mmol). The resulting mixture was stirred at reflux temperature for 3 h. To the cooled reaction was added water (200 ml) and the mixture was acidified with 1 N hydrochloric acid to pH = 4. The precipitate was filtered off, washed with water and dried in vacuo at 50 °C for 16 h which afforded 1.4 g (69 %) of 2-(hydrazinooxalyl-amino)-benzoic acid as a solid.

To a solution of the above 2-(hydrazinooxalyl-amino)-benzoic acid (1.0 g, 4.15 mmol) in methanol (20 ml) cooled to 0 °C was added potassium hydroxide (0.5 g, 8.72 mmol) and carbondisulfide (0.7 g, 9.54 mmol). The resulting mixture was stirred at reflux temperature for 6 h. To the cooled reaction was added water (100 ml) and the mixture was acidified with 1 N hydrochloric acid to pH = 1. The precipitate was filtered off, washed with water and heptane and dried in vacuo at 50 °C. The dried product (0.65 g) was purified by silica gel (400 ml) chromatography using 5 % acetic acid in ethyl acetate as eluent. Pure fractions were collected and the volatiles were evaporated in vacuo. The residue was washed with water and dried in vacuo at 50 °C for 16 h affording 0.4 g (36 %) of the title compound as a solid.

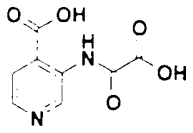
M.p.: 236 - 237 °C

Calculated for $C_{10}H_7N_3O_4S$;

C, 45.28 %; H, 2.66 %; N, 15.84 %. Found:

C, 45.48 %; H, 2.66 %; N, 15.36 %.

EXAMPLE 33



3-(Oxalyl-amino)-isonicotinic acid;

To a stirred solution of 3-amino-isonicotinic acid (0.5 g, 3.62 mmol) and triethylamine (1 ml) in dry tetrahydrofuran (50 ml) at 0 °C was added dropwise ethyl oxalyl chloride (0.5 g, 3.69 mmol). The resulting reaction mixture was stirred at room temperature for 3 h, filtered and the volatiles were evaporated in vacuo. To the residue was added water (50 ml) and the resulting mixture was extracted with diethyl ether (2 x 50 ml). The organic phase was washed with saturated aqueous sodium chloride (50 ml), dried ($MgSO_4$), filtered and the solvent evaporated in vacuo affording 0.4 g (46 %) of 3-(ethoxyoxalyl-amino)-isonicotinic acid as a solid

To a solution of the above isonicotinic acid (0.4 g, 1.7 mmol) in ethanol (25 ml) was added a solution of sodium hydroxide (141 mg, 3.53 mmol) in water (10 ml). The resulting reaction mixture was stirred at room temperature for 18 h. The volatiles were evaporated in vacuo and the residue dissolved in water (50 ml) and washed with diethyl ether (50 ml). To the aqueous phase was added 1N hydrochloric acid to pH = 1. The precipitate was filtered off and dried in vacuo at 50 °C for 18 h. The dried solid residue was washed with boiling acetone (50 ml) for 5 min. filtered off and dried in vacuo at 50 °C affording 80 mg (22 %) of the title compound as a solid.

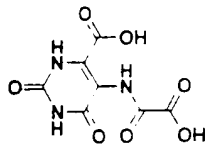
M.p.: > 250 °C;

Calculated for $C_8H_6N_2O_5$;

C, 45 72 %; H, 2.88 %; N, 13.33 %. Found:

C, 45 62 %; H, 2.98 %; N, 13.04 %.

EXAMPLE 34

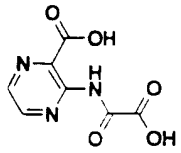


5-(Oxalyl-amino)-2,6-dioxo-1,2,3,6-tetrahydro-pyrimidine-4-carboxylic acid:

To a solution of 5-Aminoorotic acid (61.1mg, 0.36 mmol) in tetrahydrofuran (1 ml) was added imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (140 mg, 0.71 mmol) and triethylamine (50 µl, 0.36 mmol). The mixture was stirred at room temperature for 20 h. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (5.0 ml) and washed with 1 % hydrochloric acid (2 x 2 ml) then water (2 x 2 ml). The organic phase was dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residue was purified by preparative TLC (Kieselgel $60F_{254}$, 0.5 mm, hexane:ethyl acetate, 80:20) which afforded 30 mg (28 %) of 5-(*tert*-butoxyoxalyl-amino)-2,6-dioxo-1,2,3,6-tetrahydro-pyrimidine-4-carboxylic acid as a solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 (s, 9H), 7.56 (s, 2H), 8.96 (s, 1H).

5-(*tert*-Butoxyoxalyl-amino)-2,6-dioxo-1,2,3,6-tetrahydro-pyrimidine-4-carboxylic acid (28 mg, 0.094 mmol) was stirred in 20 % trifluoroacetic acid in dichloromethane (1.0 ml) at room temperature for 2 h. The reaction mixture was co-evaporated in vacuo with toluene to complete dryness which afforded 22.6 mg (100 %) of the title compound as a solid.
^{1}H NMR (400 MHz, CD_3OD) δ 7.30 (s, 2H).

EXAMPLE 35



3-(Oxalyl-amino)-pyrazine-2-carboxylic acid:

To a solution of 3-aminopyrazine-2-carboxylic acid (64.2 mg, 0.46 mmol) in tetrahydrofuran (1 ml) was added imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (181mg, 0.92 mmol) and triethylamine (64.3 µl, 0.46 mmol). The mixture was stirred at room temperature for 20 h. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (5.0 ml) and washed with 1 % hydrochloric acid (2 x 2 ml) then water (2 x 2 ml). The organic phase was dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residue was washed

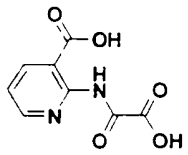
with diethyl ether (4 x 1.0 ml) affording 48 mg (39 %) of 3-(tert-butoxyoxalyl-amino)-pyrazine-2-carboxylic acid as a solid.

^{1}H NMR ($CDCl_3$ + CD_3OD) δ 1.70 (s, 9H), 8.02 (d, 1H, J = 1.5 Hz), 8.36 (d, 1H, J = 1.5 Hz).

3-(tert-Butoxyoxalyl-amino)-pyrazine-2-carboxylic acid (31.7 mg, 0.12 mmol) was stirred in 20 % trifluoroacetic acid in dichloromethane (1 ml) at room temperature for 2 h. The volatiles were evaporated in vacuo and the residue co-evaporated with toluene in vacuo affording 25 mg (100 %) of the title compound as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, 1H, J = 1.5 Hz), 8.15 (d, 1H, J = 1.5 Hz), 8.62 (s, 1H).

EXAMPLE 36



2-(Oxalyl-amino)-nicotinic acid:

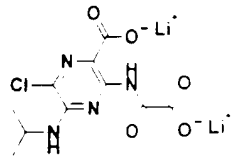
To a solution of 2-aminonicotinic acid (61.4 mg, 0.45 mmol) in tetrahydrofuran (1 ml) was added imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (174.2mg, 0.89 mmol) and triethylamine (62 µl, 0.45 mmol). The mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (5.0 ml) and washed with 1% hydrochloric acid (2 x 2 ml) then water (2 x 2 ml). The organic phase was dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residue (125 mg) was purified by preparative TLC (Kieselgel $60F_{254}$, 1mm, CH_2Cl_2/MeOH, 80/20) affording 7.9 mg (7 %) of 2-(*tert*-butoxyoxalyl-amino)-nicotinic acid as a solid.

^{1}H NMR (400 MHz, CD_3OD) δ 1.80 (s, 9H), 7.40 (m, 1H), 8.50 - 8.70 (m, 2H).

2-(*tert*-Butoxyoxalyl-amino)-nicotinic acid (7.1 mg, 0.03 mmol) was stirred in 20 % trifluoroacetic acid in dichloromethane (0.5 ml) at room temperature for 2 h. The volatiles were evaporated to dryness in vacuo affording 5.6 mg (100 %) of the title compound as a solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.40 (m, 1H), 8.50 - 8.70 (m, 2H).

EXAMPLE 37



6-Chloro-5-isopropylamino-3-(oxalyl-amino)-pyrazine-2-carboxylic acid, dilithium salt:

To a solution of 3-amino-6-chloro-5-isopropylamino-pyrazine-2-carboxylic acid (65.4 mg, 0.27 mmol) in tetrahydrofuran (1 ml) was added imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (104.8 mg, 0.54 mmol) and triethylamine (37.4 µl, 0.27 mmol). The mixture was stirred at room temperature for 20 h followed by heating to 50 °C for 1.5 h. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (5.0 ml) and washed with 1 % hydrochloric acid (2 x 2 ml) then water (2 x 2 ml). The organic phase was dried ($MgSO_4$), filtered and the solvent evaporated in vacuo affording crude 97 mg (97 %) of 3-(*tert*-butoxyoxalyl-amino)-6-chloro-5-isopropylamino-pyrazine-2-carboxylic acid as an oil.

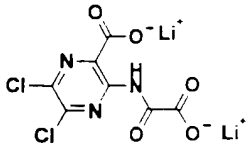
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (d, 6H), 1.80 (s, 9H), 4.10 (s, 3H), 4.40 (m, 1H).

3-(*tert*-Butoxyoxalyl-amino)-6-chloro-5-isopropylamino-pyrazine-2-carboxylic acid (30 mg, 0.1 mmol) was dissolved in tetrahydrofuran (1 ml) and 1.0 N lithium hydroxide (1 ml, 1 mmol) was added at room temperature. The reaction mixture was stirred for 3 days at room temperature. After removing the solvent in vacuo, the residue was dissolved in ethyl acetate (20 ml) and washed with water (4 x 3.0 ml). The organic phase was dried (Na_2SO_4), filtered and the solvent evaporation in vacuo affording 21 mg (82 %) of the title compound as a solid.

^{1}H NMR (400 MHz, CD_3OD) δ 1.42 (d, 6H), 4.50 (m, 1H).

MS *m/z* 228 (M-74).

EXAMPLE 38

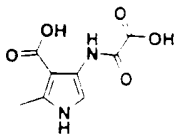


5,6-Dichloro-3-(oxalyl-amino)-pyrazine-2-carboxylic acid, dilithium salt:

To a solution of 3-amino-5,6-dichloro-pyrazine-2-carboxylic acid (57 mg, 0.26 mmol) in tetrahydrofuran (0.5 ml) was added imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (100.6 mg, 0.513 mmol) and triethylamine (35.8 µl, 0.26 mmol). The mixture was stirred at room temperature for 20 h followed by heating at 40 °C for 4 h. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (5.0 ml) and washed with 1 % hydrochloric acid (2 x 2 ml) then water (2 x 2 ml). The organic phase was dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residual oil was purified by preparative tlc (Kieselgel $60F_{254}$, 1 mm, hexane: ethyl acetate, 1:1) affording 23.6 mg (26 %) of 3-(*tert*-butoxyoxalyl-amino)-5,6-dichloro-pyrazine-2-carboxylic acid as an oil.
^1H NMR (400 MHz, $CDCl_3$) δ 1.58 (s, 9H), 1.80 (s, 9H), 3.90 (s, 3H).

To a solution of 3-(*tert*-butoxyoxalyl-amino)-5,6-dichloro-pyrazine-2-carboxylic acid (23 mg, 0.07 mmol) in tetrahydrofuran (0.5 ml) was added a1.0 N aqueous solution of lithium hydroxide (0.5 ml) and the resulting mixture was stirred for 3 days. After removing the solvent in vacuo, the residue was dissolved in ethyl acetate (20 ml) and washed with water (4 x 3.0 ml). The organic phase was dried (Na_2SO_4), filtered and the solvent evaporation in vacuo affording 14 mg (80 %) of the title compound as a solid.
MS *m/z* 290.3 (M-74).

EXAMPLE 39



2-Methyl-4-(oxalyl-amino)-1H-pyrrole-3-carboxylic acid:

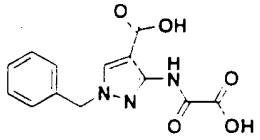
To a stirred solution of 4-(methoxyoxalyl-amino)-2-methyl-1H-pyrrole-3-carboxylic acid *tert*-butyl ester (2.0 g, 7.09 mmol) in dichloromethane (20 ml) was added trifluoro acetic acid (10 ml). The resulting reaction mixture was stirred at room temperature for 2 h. The volatiles

were evaporated in vacuo affording 1.6 g (100 %) of 4-(methoxyoxalyl-amino)-2-methyl-1H-pyrrole-3-carboxylic acid as a solid.

To a solution of the above pyrrole-3-carboxylic acid (1.2 g, 5.31 mmol) in ethanol (100 ml) was added a solution of sodium hydroxide (0.47 g, 11.7 mmol) in water (50 ml). The resulting reaction mixture was stirred at room temperature for 18 h. The volatiles were evaporated in vacuo and the residue dissolved in water (100 ml). To the aqueous phase was added concentrated hydrochloric acid to pH = 1. The suspension was washed with ethyl acetate (50 ml) and dichloromethane (50 ml) and the precipitate was filtered off and dried in vacuo at 50 °C for 2 h. The solid was dissolved in isopropanol (100 ml), filtered and evaporated in vacuo affording 0.4 g (36%) of the title compound as a solid.

Calculated for $C_8H_8N_2O_5$, 0.1 x H_2O ;
C, 44.91 %; H, 3.86 %; N, 12.98 %. Found:
C, 45.06 %; H, 3.89 %; N, 12.72 %.

EXAMPLE 40



1-Benzyl-3-(oxalyl-amino)-1H-pyrazole-4-carboxylic acid;

To a stirred solution of 3-amino-1H-pyrazole-4-carboxylic acid ethyl ester (5.0 g, 0.032 mol) and triethylamine (9 ml) in dry tetrahydrofuran (150 ml) at 0 °C was added dropwise ethyl oxalyl chloride (5.3 g, 0.039 mol). The resulting reaction mixture was stirred at room temperature for 18 h. An additional portion of ethyl oxalyl chloride (5.3 g, 0.039 mol) was added dropwise and the reaction mixture was stirred at room temperature for an additional 18 h. The volatiles were evaporated in vacuo and the residue dissolved in a mixture of water (200 ml) and ethyl acetate (200 ml). Undissolved matter was filtered off and dried in vacuo at 50

°C for 18 h affording 4.0 g (49 %) of 3-(ethoxyoxalyl-amino)-1H-pyrazole-4-carboxylic acid ethyl ester as a solid. The organic phase separated and washed with saturated aqueous sodium chloride (100 ml), dried ($MgSO_4$), filtered and the solvent evaporated in vacuo affording 3.7 g (45%) of 3-(ethoxyoxalyl-amino)-1H-pyrazole-4-carboxylic acid ethyl ester as a solid. A total yield of 7.7 g (94 %) was obtained.

To a solution of the above pyrazole-4-carboxylic acid ethyl ester (3.7 g, 0.015 mol) in dry N,N-dimethylformamide (75 ml) was added sodium hydride (640 mg, 0.016 mol, 60 % in mineral oil). The resulting reaction mixture was stirred at room temperature for 0.5 h. To the reaction mixture was added benzyl bromide (2.7 g, 0.016 mol) and the mixture was stirred at 50 °C for 4 h. Water (100 ml) was added and the reaction mixture was extracted with diethyl ether (2 x 100 ml). The combined organic extracts were washed with water (100 ml) saturated aqueous sodium chloride (2 x 50 ml), dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residue (3.8 g) was purified on silicagel (800 ml) using a mixture of ethyl acetate and heptane (1:1) as eluent. Pure fractions were collected and the solvent evaporated in vacuo affording 0.9 g (18 %) of 1-benzoyl-3-(ethoxyoxalyl-amino)-1H-pyrazole-4-carboxylic acid ethyl ester as a solid.

Unpure fraction were collected and the solvent evaporated in vacuo. The residue (1.0 g) was crystallised from diethyl ether (30 ml), filtered off and dried in vacuo at 50 °C for 2 h affording 0.9 g (18 %) of 1-benzoyl-3-(ethoxyoxalyl-amino)-1H-pyrazole-4-carboxylic acid ethyl ester as a solid. A total yield of 1.8 g (36 %) were collected.

To a solution of the above 1H-pyrazole-4-carboxylic acid ethyl ester (0.9 g, 2.61 mmol) in ethanol (50 ml) was added a solution of sodium hydroxide (0.26 g, 6.51 mmol) in water (25 ml). The resulting reaction mixture was stirred at room temperature for 60 h. The volatiles were evaporated in vacuo and the residue dissolved in water (100 ml). To the aqueous phase was added concentrated hydrochloric acid to pH = 1. The precipitate was filtered off and dried in vacuo at 50 °C for 18 h. affording 0.55 g (73 %) of the title compound as a solid.

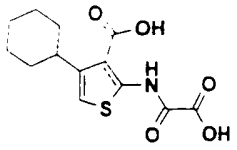
M.p.: 189 - 191 °C:

Calculated for $C_{13}H_{11}N_3O_5$, 1.75 x H_2O:

C, 48.68 %; H, 4.56 %; N, 13.10 %. Found:

C, 48.81 %; H, 4.17 %; N, 12.84 %.

EXAMPLE 41



4-Cyclohexyl-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

To a solution of 4-cyclohexyl-2-(ethoxyoxalyl-amino)-thiophene-3-carboxylic acid (60 mg, 0.18 mmol) in ethanol (10 ml) was added a solution of 1N sodium hydroxide (0.5 ml) in water (5 ml). The resulting reaction mixture was stirred at room temperature for 18 h. To the reaction mixture was added concentrated hydrochloric acid to pH = 1. The precipitate was filtered off and dried in vacuo at 50 °C for 18 h. affording 30 mg (55 %) of the title compound as a solid.

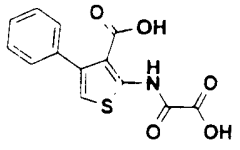
M.p.: > 250 °C:

Calculated for $C_{13}H_{15}NO_5S$, 1.5 x H_2O;

C, 48.14 %; H, 5.59 %; N, 4.32 %. Found:

C, 47.84 %; H, 9.92 %; N, 4.21 %.

EXAMPLE 42



2-(Oxalyl-amino)-4-phenyl-thiophene-3-carboxylic acid;

To a solution of 4-phenyl-2-(ethoxyoxalyl-amino)-thiophene-3-carboxylic acid ethyl ester (2.2 g, 6.33 mmol) in ethanol (50 ml) was added sodium hydroxide (630 mg, 15.83 mmol) in water (25 ml). The resulting reaction mixture was stirred at room temperature for 18 h., the volatiles were evaporated in vacuo and the residue was dissolved in water (100 ml) and washed with diethyl ether (2 x 100 ml). To the aqueous phase was added concentrated hydrochloric acid to pH = 1 and the resulting mixture was extracted with diethyl ether (2 x 100 ml). The combined organic phases were washed with saturated aqueous sodium chloride (100 ml), dried ($MgSO_4$), filtered and evaporated in vacuo affording 0.8 g of a mixture of mono ethyl ester and title compound according to NMR. The product mixture was dissolved in a mixture of ethanol (40 ml), water (20 ml) and sodium hydroxide (400 mg) and the resulting mixture was stirred at room temperature for 18 h, the volatiles were evaporated in vacuo and the residue was dissolved in water (50 ml) and washed with diethyl ether (50 ml). To the aqueous phase was added concentrated hydrochloric acid to pH = 1 and the precipitate was filtered off, washed with diethyl ether and dissolved in 2-propanol (25 ml). Undissolved matter was filtered off and the organic phase evaporated in vacuo affording 180 mg (10 %) of the title compound as a solid.

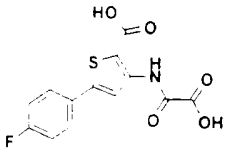
M.p.: 196 - 198 °C.

Calculated for $C_{13}H_9NO_5S$, 0.5 H_2O;

C, 52.00 %; H, 3.36 %; N, 4.66 %. Found:

C, 52.21 %; H, 3.44 %; N, 4.50 %.

EXAMPLE 43



5-(4-Fluoro-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid;

A solution of 5-(4-fluorophenyl)-3-aminothiophene-2-carboxylic acid methyl ester (1.0 g, 4.0 mmol) and triethylamine (11.1, 80 mmol) in dry tetrahydrofuran (40 ml) was cooled on ice and dropwise added ethyl oxalyl chloride (1.2 g, 9.0 mmol). After stirring for 2h, the reaction mixture was filtered and the solvent evaporated in vacuo. The residue was dissolved in dichloromethane, washed with 0.1 N hydrochloric acid (2 x-dicared). The organic phase was

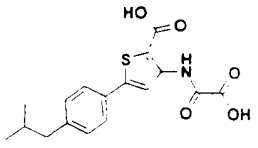
dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residue was submitted to flash chromatography using toluene/ethyl acetate (19:1) as eluent, to give 1.19 g (85 %) of 5-(4-fluorophenyl)-3-(ethoxyoxalylamino)-thiophene-2-carboxylic acid ethyl ester.

To a solution of 5-(4-fluorophenyl)-3-(ethoxyoxalylamino)-thiophene-2-carboxylic acid ethyl ester (1.19 g, 3.4 mmol) in methanol (150 ml) was added 2 N sodium hydroxide (20 ml). The reaction mixture was stirred at 60 °C for 18 h. The volatiles were evaporated in vacuo. The residue was added water and 1N hydrochloric acid (pH = 1), and the product extracted with a mixture of dichloromethane/2-propanol. The organic phases were dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The product was recrystallised from methanol/water to give 619 mg (67 %) of the title compound as a solid.

Calculated for $C_{13}H_8FNO_5S$, 0.5 H_2O;
C, 49.06 %; H, 2.83%; N, 4.40 %. Found:
C, 49.06 %; H, 2.72%; N, 4.31%.

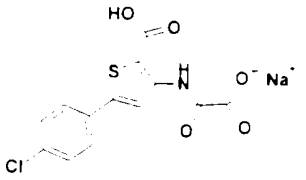
In a similar way as described in Example 43 the following compounds were prepared.

EXAMPLE 44



5-(4-Isobutyl-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid;
Calculated for $C_{17}H_{17}NO_5S$, 0.33 x H_2O;
C, 57.79 %; H, 5.00 %; N, 3.96 %. Found:
C, 57.79 %; H, 5.08 %; N, 3.89 %.

EXAMPLE 45



5-(4-Chloro-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid, mono sodium salt;

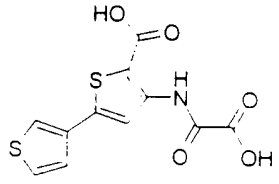
M.p.: > 250 °C;

Calculated for $C_{13}H_7ClNO_5SNa$, 1x H_2O;

C, 42.63 %; H, 2.52 %; N, 3.55 %. Found:

C, 42.69 %; H, 2.48 %; N, 3.83 %.

EXAMPLE 46



4-(Oxalyl-amino)-[2,3]-bithiophenyl-5-carboxylic acid;

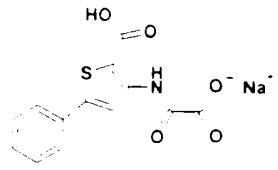
M.p.: 220 - 222 °C;

Calculated for $C_{11}H_7NO_5S_2$;

C, 44.44 %; H, 2.37 %; N, 4.71 %. Found:

C, 44.17 %; H, 2.43 %; N, 4.54 %.

EXAMPLE 47



3-(Oxalyl-amino)-5-phenyl-thiophene-2-carboxylic acid, mono sodium salt;

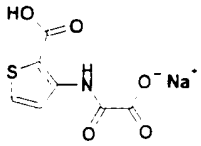
M.p.: > 250 °C:

Calculated for $C_{13}H_8NO_5SNa$, 1.6 x H_2O;

C, 45.64 %; H, 3.30 %; N, 4.09 %. Found:

C, 45.25 %; H, 2.93 %; N, 3.92 %.

EXAMPLE 48



3-(Oxalyl-amino)-thiophene-2-carboxylic acid, mono sodium salt;

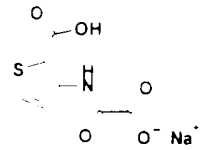
M.p.: > 250 °C:

Calculated for $C_7H_7NO_5SNa$, 1.5 x H_2O;

C, 31.83 %; H, 2.67 %; N, 5.30 %. Found:

C, 32.23 %; H, 3.14 %; N, 5.15 %.

EXAMPLE 49



4-Methyl-3-(oxalyl-amino)-thiophene-2-carboxylic acid, mono sodium salt;

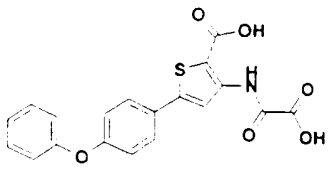
M.p.: 232 - 234 °C.

Calculated for $C_8H_6NO_5SNa$, 1.5 x H_2O;

C, 34.54 %; H, 3.26 %; N, 5.03 %. Found:

C, 34.58 %; H, 3.30 %; N, 4.81 %.

EXAMPLE 50



3-(Oxalyl-amino)-5-(4-phenoxy-phenyl)-thiophene-2-carboxylic acid

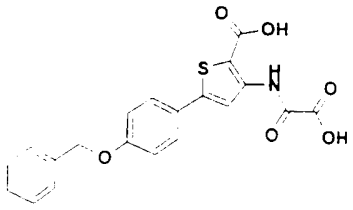
M.p.: 230 °C (decomp.)

Calculated for $C_{19}H_{13}NO_6S$, 1.25 x H_2O

C, 56.22 %; H, 3.85 %; N, 3.45 %. Found:

C, 56.00 %; H, 3.57 %; N, 3.39 %.

EXAMPLE 51



5-(4-Benzyloxy-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid

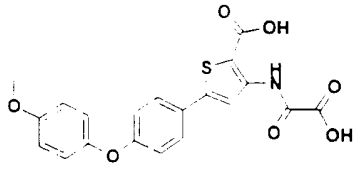
M.p.: 210 °C (decomp.)

Calculated for $C_{20}H_{15}NO_6S$

C, 60.45 %; H, 3.80 %; N, 3.52 %. Found:

C, 59.94 %; H, 3.79 %; N, 4.45 %.

EXAMPLE 52



5-(4-(4-Methoxy-phenoxy)-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid

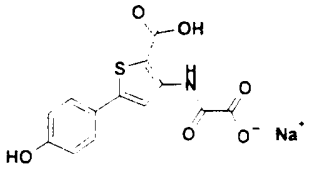
M.p.: 215 °C (decomp.)

Calculated for $C_{20}H_{15}NO_7S$, 1.5 H_2O

C, 54.54 %; H, 4.12 %; N, 3.18 %. Found:

C, 54.80 %; H, 3.88 %; N, 3.15 %.

EXAMPLE 53



5-(4-Hydroxy-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid, mono sodium salt

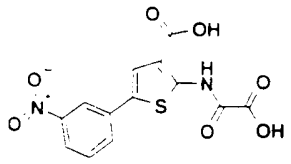
M.p.: 205 - 206 °C

Calculated for $C_{13}H_9NO_6SNa_1$, 0.75 x H_20

C, 45.42 %; H, 3.08 %; N, 4.07 %. Found:

C, 45.11 %; H, 3.16 %; N, 3.98 %.

EXAMPLE 54



5-(3-Nitro-phenyl)-2-(oxalyl-amino)-thiophene-3-carboxylic acid:

To 3-nitrophenethyl alcohol (102 mg, 0.61 mmol) in dichloromethane (2.2 ml) at room temperature under nitrogen was added a solution of Dess-Martin periodinane reagent (285 mg, 0.67 mmol) in dichloromethane (2.7 ml). The reaction was stirred at room temperature under nitrogen for 45 minutes, at which time tlc analysis (hexane/ethyl acetate, 50/50) indicated the reaction was complete. Diethyl ether (5.0 ml) was added followed by a solution of 10 % sodium sulfite/saturated sodium bicarbonate (1:1, 5.0 ml). The emulsion gradually turned to a clear heterogeneous solution after standing for 10 minutes. Additional dichloromethane was added and the organic phase was washed with water (5 ml), dried ($MgSO_4$), filtered and evaporated in vacuo which afforded 100 mg (100 %) of 3-nitrophenyl-acetaldehyde as a clear oil. The aldehyde was used without further purification in the next step.
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (s, 2H), 7.65 (d, 2H), 8.20 (s, 1H), 8.25 (m, 1H), 9.90 (s, 1H).

A mixture of *tert*-butyl cyanoacetate (67 mg, 0.48 mmol), 3-nitrophenyl acetaldehyde (86 mg, 0.52 mmol), triethylamine (73 µl, 0.52 mmol) and elemental sulfur (17 mg, 0.52 mmol) in N,N-dimethylformamide (0.5 ml) was stirred at 60 °C for 1.5 h. After cooling to room temperature, the dark solution was diluted with ethyl acetate and washed with water (3 x 5 ml). The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated in vacuo which afforded crude 2-amino-5-(3-nitro-phenyl)-thiophene-3-carboxylic acid *tert*-butyl ester (191 mg). Purification by preparative TLC (hexane/ethyl acetate, 80/20) afforded 74 mg (49 %) of 2-amino-5-(3-nitro-phenyl)-thiophene-3-carboxylic acid *tert*-butyl ester as a solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 9H), 6.05 (s, 2H), 7.20 (s, 1H), 7.40 (t, 1H), 7.68 (d, 1H), 7.90 (d, 1H), 8.25 (s, 1H).

A solution of 2-amino-5-(3-nitro-phenyl)-thiophene-3-carboxylic acid *tert*-butyl ester (66 mg, 0.21 mmol), imidazol-1-yl-oxoacetic acid *tert*-butyl ester (202 mg, 1.03 mmol) and triethylamine (40.4 µl, 0.21 mmol) in tetrahydrofuran (0.5 ml) was stirred at room temperature for 3 h. The volatiles were evaporated in vacuo and the residue was dissolved in ethyl acetate and washed successively with water (3 x 5 ml) and brine (5ml). The organic layer was dried (Na_2SO_4), filtered and the solvent evaporated in vacuo affording crude product. Purification by preparative TLC gave 91 mg (98 %) of 2-(*tert*-butoxyoxalyl-amino)-5-(3-nitrophenyl)-thiophene-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 9H), 1.62 (s, 9H), 7.5 (s, 1H), 7.55 (t, 1H, J = 8.4 Hz), 7.84 (d, 2H, J = 8.4 Hz), 8.16 (d, 1H, J = 8.4 Hz), 8.45 (s, 1H).

MS *m/z*: 447 (M-1).

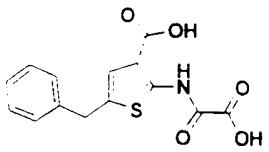
The above 3-nitrophenyl-thiophene (85 mg, 0.19 mmol) was dissolved in a 20 % solution of trifluoroacetic acid in dichloromethane (3.0 ml) and stirred at room temperature for 6 h. The solution was co-evaporated in vacuo with toluene affording 64 mg (100 %) of the title compound.

^{1}H-NMR (400 MHz, CD_3OD) δ 7.71 (t, 1H, J = 8.25 Hz), 7.8 (s, 1H), 8.1 (d, 1H, J = 7.5 Hz), 8.2 (d, 1H, J = 9 Hz), 7.86 (m, 1H).

MS *m/z*: 335 (M-1).

The following examples were prepared in a similar way as described in Example 54.

EXAMPLE 55



2-(Oxalyl-amino)-5-(phenyl-methyl)thiophene-3-carboxylic acid

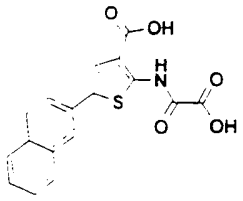
M.p.: 230 - 231 °C

Calculated for $C_{14}H_{11}NO_5S$.

C, 54.89 %; H, 3.63 %; N, 4.40 %. Found:

C, 54.94 %, H, 3.63 %; N, 4.43 %.

EXAMPLE 56

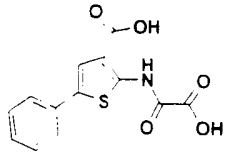


5-(Naphtnalen-2-yl)-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 7.42 - 7.49 (m, 2H), 7.66 (d, 1H, J = 4.5 Hz), 7.75 (m, 1H), 7.8 - 7.9 (m, 3H), 8.04 (d, 1H ,J = 7.5 Hz).

MS *m/z*: 340 (M-1).

EXAMPLE 57



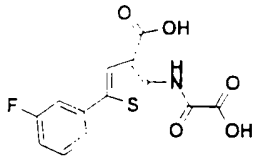
2-(Oxalyl-amino)-5-phenyl-thiophene-3-carboxylic acid;

M.p.: 238 - 240 °C

^{1}H NMR (400 MHz, CD_3OD) δ 7.3 (t, 1H, J = 4.5 Hz), 7.38 (t, 1H, J = 4.5Hz), 7.54 (s, 1H), 7.61 (m, 3H).

Calculated for $C_{13}H_9NO_5S$, 1 x H_2O;
C, 47.13 %; H, 3.04 %; N, 4.23 %. Found:
C, 47.34 %; H, 3.53 %; N, 4.20 %.

EXAMPLE 58

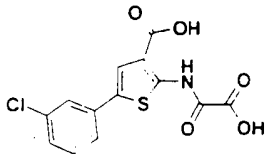


5-(2-Fluoro-phenyl)-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 7.18 - 7.23 (m, 2H), 7.30 (m, 1H), 7.63 -7.69 (m, 2H).

MS *m/z*: 308 (M-1).

EXAMPLE 59



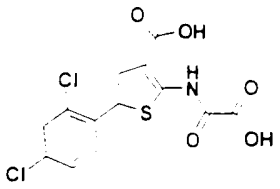
5-(3-Chloro-phenyl)-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

Yield: 99 %.

^{1}H NMR (400 MHz, CD_3OD) δ 7.28 (m, 1H), 7.38 (m, 1H), 7.52 - 7.61 (m, 3H).

MS *m/z*: 324 (M-1).

EXAMPLE 60

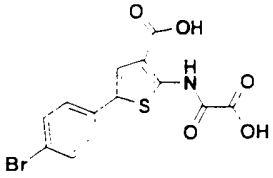


5-(2,4-Dichloro-phenyl)-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 7.37 (m, 1H), 7.39 (m, 1H), 7.52-7.58 (m, 3H).

MS *m/z* 358 (M-1).

EXAMPLE 61

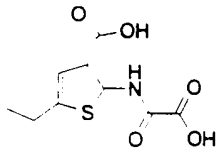


5-(4-Bromo-phenyl)-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (s, 4H), 7.54 (s, 1H).

MS *m/z* 370 (M-1).

EXAMPLE 62

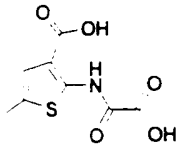


5-Ethyl-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 1.35 (t, 3H, J = 3.75), 2.95 (q, 2H), 7.05 (s, 1H).

MS *m/z*. 170.2 (M-73) (-COCOOH), 228.1 (M-1).

EXAMPLE 63

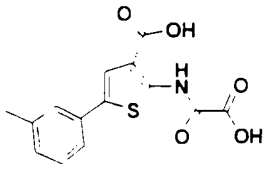


5-Methyl-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 2.6 (s, 3H), 7.05 (s, 1H).

MS *m/z*: 228 (M-1).

EXAMPLE 64

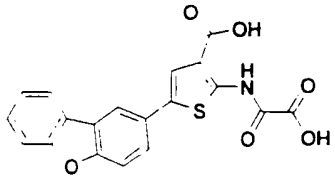


5-(3-Methyl-phenyl)-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 2.39 (s, 3H), 7.12 (d, 1H, J = 8 Hz), 7.25 (t, 1H, J = 7.5 Hz), 7.4 (m, 2H), 7.5 (s, 1H).

MS *m/z* 304, 232 (M-1).

EXAMPLE 65

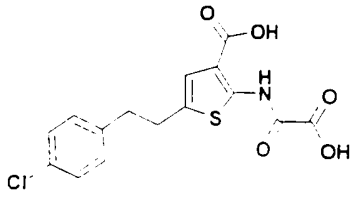


5-Dibenzofuran-2-yl-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.4 (t, 1H, J = 2 Hz), 7.52 (t, 1H J = 2 Hz), 7.7 (m, 3H), 7.9 (t, 1H, J = 2 Hz), 8.25 (d, 1H, J = 2 Hz), 8.5 (s, 1H).

MS *m/z* 380.5 (M-1).

EXAMPLE 66



5-(2-(4-Chloro-phenyl)-ethyl)-2-(oxalyl-amino)-thiophene-3-carboxylic acid, mono sodium salt

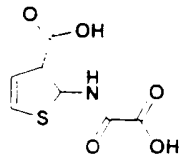
M.p.: > 250 °C

Calculated for $C_{15}H_{11}N_1Cl_1O_5S_1Na_1$, 0.75 x H_2O

C, 46.28 %; H, 3.24 %; N, 3.60 %. Found:

C, 46.17 %; H, 3.38 %; N, 3.40 %.

EXAMPLE 67



2-(Oxalyl-amino)-thiophene-3-carboxylic acid;

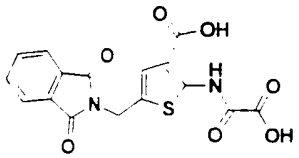
M.p : 225 - 228 °C

Calculated for $C_7H_5N_1O_5S_1$, 1.25 x H_2O

C, 35.37 %; H, 3.18 %; N, 5.89 %. Found:

C, 35.53 %; H, 2.82 %; N, 5.72 %.

EXAMPLE 68



5-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(oxalyl-amino)thiophene-3-carboxylic acid;

To a stirred mixture at 0 °C of 2-(3-hydroxy-propyl)-isoindole-1,3-dione (0.2 g, 0.97 mmol), 0.7 N sodium bromide (0.70 ml, 0.46 mmol), 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical) TEMPO (3.0 mg, 0.02 mmol) in dichloromethane (1 ml) was added dropwise a solution of bleach (2.1 ml, 4.9 mmol) and sodium hydrogencarbonate (117 mg, 1.4 mmol). The mixture was stirred at 0 °C for 2 h after the addition was finished. The mixture was extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were washed with 10% sodium thiosulfate (3 x 10 ml), brine (10 ml), dried ($MgSO_4$), filtered and the solvent was evaporated in vacuo. The residue was washed with ethyl acetate (2 x 1 ml) affording after drying 161 mg (81 %) of 3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propionaldehyde as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.85 (dd, 2H, J = 5.6, 2.8 Hz), 7.73 (dd, 2H, J = 5.6, 2.8 Hz), 4.04 (t, 2H, J = 7.2 Hz), 2.89 (t, 2H, J = 7.2 Hz).

To a solution of the above aldehyde (150 mg, 0.74 mmol), triethylamine (113 ml, 0.81 mmol) and sulfur (24 mg, 0.81 mmol) in dichloromethane (10 ml) at room temperature was added *tert*-butyl cyanoacetate (114 mg, 0.81 mmol). The mixture was stirred and heated at reflux temperature under nitrogen for 2 h. After cooled to room temperature the precipitate was filtered off affording 189 mg of 2-amino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-thiophene-3-carboxylic acid *tert*-butyl ester as a solid.

The filtrate was evaporated in vacuo, the residue was taken into ethyl acetate (50 ml), washed with 0.5 N hydrochloric acid (2 x 10 ml), saturated sodium bicarbonate (2 x 10 ml),

brine (10 ml), dried ($MgSO_4$) and filtered. The solvent was evaporated in vacuo and the residue was washed with cold ethyl acetate (2 x 1 ml) affording 52 mg of 2-amino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-thiophene-3-carboxylic acid *tert*-butyl ester as a solid. A total yield of 241 mg (91 %) was obtained.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (dd, 2H, J = 7.2, 4 Hz), 7.72 (dd, 2H, J = 7.2, 4 Hz), 6.97 (s, 1H), 5.83 (s, 2H, NH_2), 4.78 (s, 2H), 1.56 (s, 9H)

To a stirred solution of the above thiophene (100 mg, 0.28 mmol) in tetrahydrofuran (2 ml) was added a solution of imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (60 mg, 0.31 mmol) in tetrahydrofuran (1 ml). The mixture was stirred at room temperature for 3 h. The solvent was evaporated in vacuo. The residue was dissolved in ethyl acetate (50 ml), washed with 0.5 N hydrochloric acid (2 x 5 ml), saturated sodium bicarbonate (2 x 5 ml), brine (5 ml), dried ($MgSO_4$) and filtered. The solvent was evaporated in vacuo affording 130 mg (96 %) of 2-(*tert*-butoxyoxalyl-amino)-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-thiophene-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.23 (s, 1H), 7.87 (dd, 2H, J = 7.2, 4 Hz), 7.73 (dd, 2H, J = 7.2, 4 Hz), 7.24 (s, 1H), 4.93 (s, 2H), 1.60 (s, 9H), 1.57 (s, 9H).

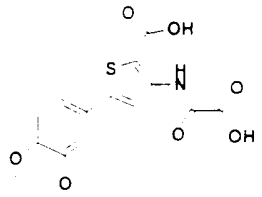
To a solution of trifluoroacetic acid (1 ml) in dichloromethane (1 ml) was added the above di-*tert*-butyl ester (100 mg, 0.21 mmol). The solution was stirred at room temperature for 1 h. The solvent was evaporated in vacuo. The residue was washed with dichloromethane (3 x 1 ml) which afforded 63 mg (82 %) of the title compound as a solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s, 1H), 7.89 (m, 2H), 7.87 (m, 2H), 7.10 (s, 1H), 4.83 (s, 2H).

MS *m/z*: 373 (M-1).

In a similar way as described in Example 43 the following compounds were prepared.

EXAMPLE 69



5-(3,4-Dimethoxy-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid

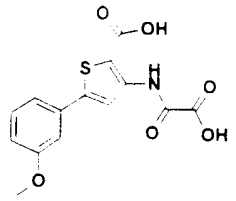
M.p.: 230 - 231 °C

Calculated for $C_{15}H_{13}N_1O_7S_1$, 1 x H_2O

C, 48.78 %; H, 4.09 %; N, 3.79 %. Found:

C, 49.01 %; H, 3.75 %; N, 3.79 %.

EXAMPLE 70



5-(3-Methoxy-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid

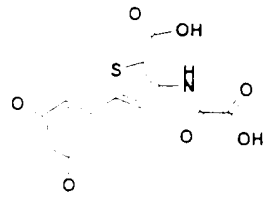
M.p.: 217 - 218 °C

Calculated for $C_{14}H_{11}N_1O_6S_1$, 0.75 x H_2O

C, 50.22 %; H, 3.76 %; N, 4.18 %. Found:

C, 50.02 %; H, 3.73 %; N, 4.16 %.

EXAMPLE 71



5-(3,5-Dimethoxy-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid

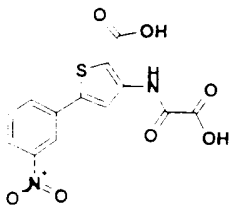
M.p.: 223 - 225 °C

Calculated for $C_{15}H_{13}N_1O_7S_1$, 1.25 x H_2O

C, 48.19 %; H, 4.18 %; N, 3.75 %. Found:

C, 48.25 %; H, 4.10 %; N, 3.39 %.

EXAMPLE 72



5-(3-Nitro-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid

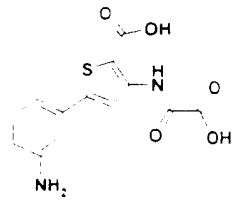
M.p.: > 250 °C

Calculated for $C_{13}H_7N_1O_7S_1Na_1$, 1.25 x H_2O

C, 41.01 %; H, 2.51 %; N, 7.36 %. Found:

C, 41.03 %; H, 2.38 %; N, 7.17 %.

EXAMPLE 73



5-(3-Amino-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid

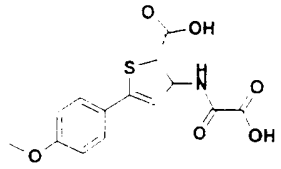
M.p.: > 250 °C

Calculated for $C_{13}H_{10}N_2O_5S_1$, 0.5 x H_20

C, 49.52 %; H, 3.52 %; N, 8.88 %. Found:

C, 49.48 %; H, 3.44 %; N, 8.71 %.

EXAMPLE 74



5-(4-Methoxy-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid

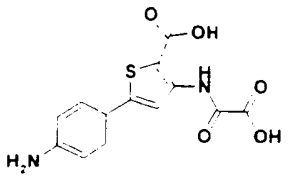
M.p.: 220 - 221 °C

Calculated for $C_{14}H_{11}N_1O_6S_1$, 0.4 x H_20

C, 51.19 %; H, 3.62 %; N, 4.62 %. Found:

C, 51.29 %; H, 3.53 %; N, 3.96 %.

EXAMPLE 75



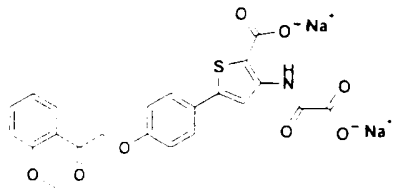
5-(4-Amino-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid;

Calculated for $C_{13}H_{10}N_2O_5S_1$, 0.5 x H_2O

C, 49.52 %; H, 3.52 %; N, 8.88 %. Found:

C, 49.40 %; H, 3.87 %; N, 8.23 %

EXAMPLE 76



5-(4-(2-(2-Methoxy-phenyl)-2-oxo-ethoxy)-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid, disodium salt

To a solution of 3-(ethoxyoxalylamino)-5-(4-hydroxyphenyl)thiophene-2-carboxylic acid methyl ester (524 mg, 1.5 mmol) and potassium carbonate (275 mg, 2.0 mmol) in N,N-dimethylformamide (35 ml) was under an nitrogen atmosphere added ω-brom-2-methoxyacetophenon (460 mg, 2.0 mmol). After stirring for 3 h, the precipitate crude 3-(ethoxyoxalylamino)-5-(4-(2-(2-methoxyphenyl)-2-oxy-ethoxy)phenyl)-thiophene-2-carboxylic acid methyl ester (1.0 g) was filtered off.

To a solution of crude 3-(ethoxyoxalylamino)-5-(4-(2-(2-methoxyphenyl)-2-oxy-ethoxy)phenyl)-thiophene-2-carboxylic acid methyl ester (0.5 g) in methanol (15 ml) was added 1N sodium hydroxide (10 ml). After stirring at 65 °C for 3h, the product was isolated by filtration and washed with a mixture of water and ethanol (1:1) affording after drying 290 mg of the title compound as a solid.

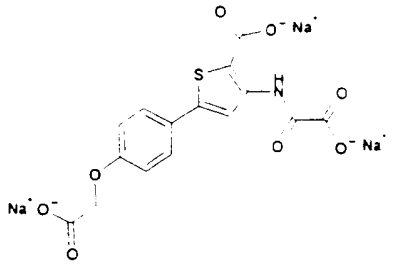
M.p.: 286 - 287 °C.

Calculated for $C_{22}H_{18}N_1O_9S_1Na_2$;

C, 50.19 %; H, 3.42 %; N, 2.66 %. Found:

C, 51.18 %; H, 3.42 %; N, 2.58 %.

EXAMPLE 77



5-(4-Carboxymethoxy-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid, trisodium salt;

To a solution of 3-(ethoxyoxalylamino)-5-(4-hydroxyphenyl)thiophene-2-carboxylic acid methyl ester (307 mg, 1.0 mmol) and potassium carbonate (166 mg, 1.2 mmol) in N,N-dimethylformamide (5 ml) was added 2-bromoacetamide (165 mg, 1.2 mmol). After stirring at 50 °C for 16 h, the reaction mixture was quenched by addition of water, and the precipitate 5-(4-carbamoylmethoxy-phenyl)-3-(ethoxyoxalylamino)-thiophene-2-carboxylic acid methyl ester (70 mg) was isolated by filtration.

The pH of the filtrate was adjusted to 1-2 with 1 N hydrochloric and the semi hydrolysed product, 5-(4-carbamoylmethoxy-phenyl)-3-(oxalylamino)-thiophene-2-carboxylic acid methyl ester (300 mg), was isolated by filtration. To a suspension of 5-(4-carbamoylmethoxy-phenyl)-3-(oxalylamino)-thiophene-2-carboxylic acid methyl ester (295 mg, 0.78 mmol) in methanol (5 ml) and water (5 ml) was added 1 N sodium hydroxide (2 ml). After stirring for 5 days the precipitate was filtered of affording 105 mg (88 %) of the title compound as a solid.

M.p.: > 300 °C.

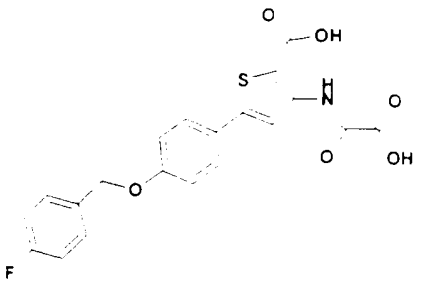
Calculated for $C_{15}H_{12}N_1O_{10}S_1Na_3$:

C, 38.56 %; H, 2.59 %; N, 3.00 %. Found:

C, 38.73 %; H, 2.74 %; N, 3.06 %.

In a similar way as described in Example 77 the following compound was prepared:

EXAMPLE 78

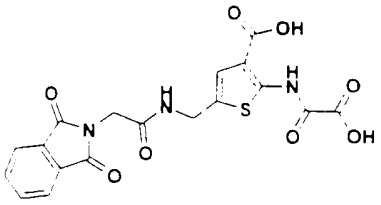


5-(4-(4-Fluoro-benzyloxy)-phenyl)-3-(oxalyl-amino)-thiophene-2-carboxylic acid;

^{1}H NMR (300 MHz, DMSO-d_6) δ 5.15 (s, 2H), 7.1 (d, 2H), 7.25 (t, 2H), 7.55 (q, 2H), 7.7 (d, 2H), 8.2 (s, 1H).

SP/MS: 415 (M+, 12%), 372, 353, 299, 218, 190, 162, 109 (100%).

EXAMPLE 79



5-((2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-acetylamino)-methyl)-2-(oxalyl-amino)-thiophene-3-carboxylic acid;

To a solution of 2-(*tert*-butoxyoxalyl-amino)-5-(1,3-dioxo-1,3-dihydro-isoindol-2-yl-methyl)-thiophene-3-carboxylic acid *tert*-butyl ester (0.4 g, 0.82 mmol, prepared as described in example 30) in dichloromethane (2 ml) was added anhydrous hydrazine (28 ml, 0.9 mmol) and the mixture stirred at ambient temperature for 19 h under nitrogen. An additional portion of hydrazine (84 ml, 2.7 mmol) and dichloromethane (5.5 ml) was added and stirring was continued for an additional 88 h. Dichloromethane (50 ml) was added and the reaction mixture was placed in a sonicator for 20 min and filtered through Celite. The filtrate was evaporated in vacuo affording 0.24 g (82 %) of 5-aminomethyl-2-(*tert*-butoxyoxalyl-amino)-thiophene-3-

carboxylic acid *tert*-butyl ester as a solid which was used without further purification in the next step.

To a solution of (1,3-dioxo-1,3-dihydro-isoindol-2-yl)-acetic acid (0.17 g, 0.82 mmol), 1-hydroxybenzotriazole (0.133 g, 0.98 mmol) and 2,6 lutidine (0.4 ml) in dry acetonitrile (10 ml) under nitrogen cooled in an ice bath was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.21 g, 1.1 mmol) and the solution was stirred for 0.5 h. 5-Aminomethyl-2-(*tert*-butoxyoxalyl-amino)-thiophene-3-carboxylic acid *tert*-butyl ester (0.24 g, 0.68 mmol) was added, the cooling bath removed, and the solution stirred at ambient temperature for 20 h. The volatiles were evaporated in vacuo and the residue dissolved in dichloromethane and washed with saturated aqueous sodium bicarbonate and 1N hydrochloric acid, dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue (0.18 g) was dissolved in dry tetrahydrofuran (6 ml) under nitrogen, imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (0.25 g, 1.3 mmol) was added and the solution stirred at ambient temperature for 17 h, the solvent evaporated in vacuo and the residue dissolved in a mixture of dichloromethane and saturated aqueous sodium bicarbonate solution. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue was subjected to chromatography on silica gel affording 0.1 g of 2-(*tert*-butoxyoxalyl-amino)-5-((2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-acetylamino)-methyl)-thiophene-3-carboxylic acid *tert*-butyl ester.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.3 (bs, 1H), 7.9 (m, 2H), 7.8 (m, 2H), 7.1 (s, 1H), 6.5 (m, 1H), 4.6 (m, 2H), 4.4 (s, 2H,), 1.8 (s, 9H), 1.6 (s, 9H).

To 2-(*tert*-butoxyoxalyl-amino)-5-((2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-acetyl-ami-no)-methyl)-thiophene-3-carboxylic acid *tert*-butyl ester (0.1 g, 0.18 mmol) was added 20 % trifluoroacetic acid in dichloromethane (4 ml) and the reaction mixture was stirred at ambient temperature under nitrogen for 14 h. The volatiles were evaporated in vacuo and the residue chased with dichloromethane until a solid remained. The precipitate was filtered off and dried in vacuo for 18 h affording in quantitative yield the title compound as a solid.

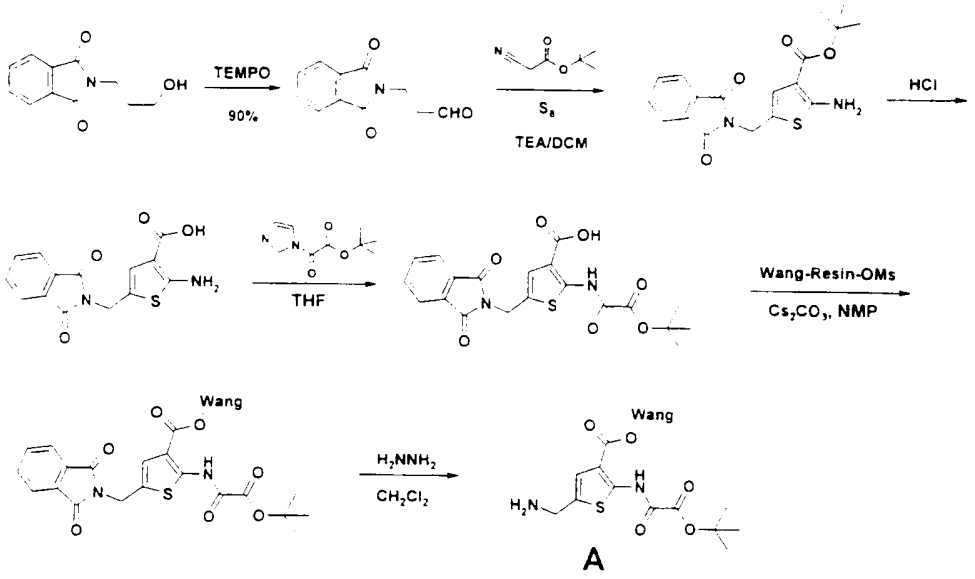
Mp. 243 – 244 °C (dec).

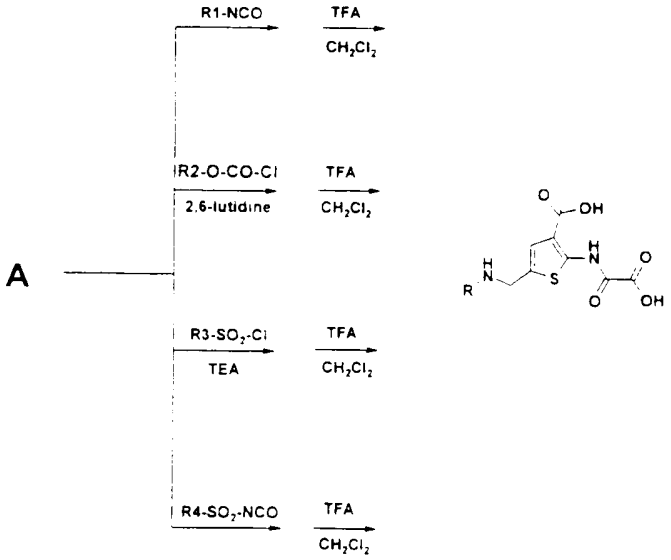
MS *m/z*: 430 (M-1).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.1 (s, 1H), 8.9 (s, 1H), 7.8 – 7.9 (m, 4H), 7.1 (s, 1H), 4.4 (m, 2H), 4.2 (s, 2H).

EXAMPLE 80

Using a solid phase chemistry approach a 64 member library was synthesised according to the following scheme

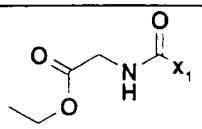
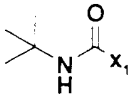
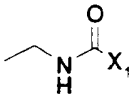
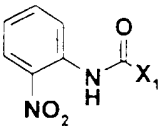
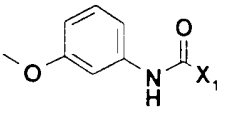
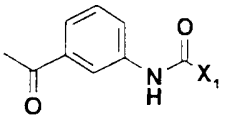
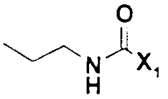
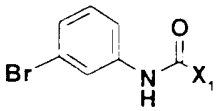
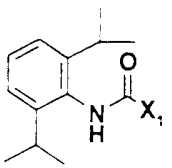


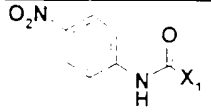
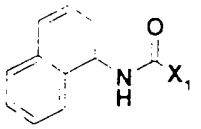
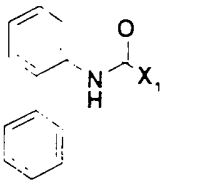
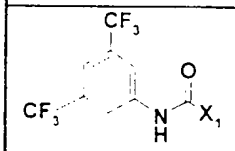
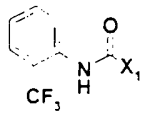
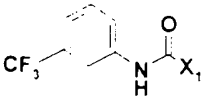
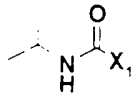
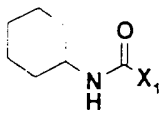
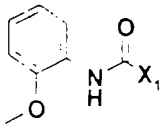
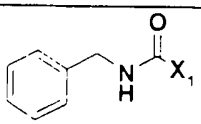


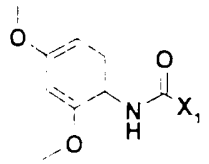
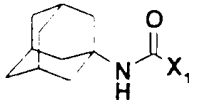
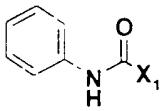
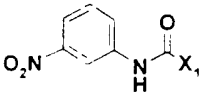
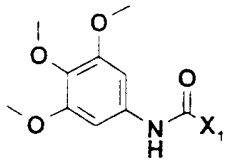
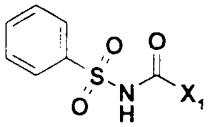
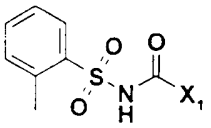
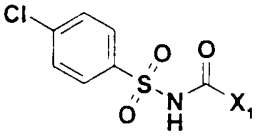
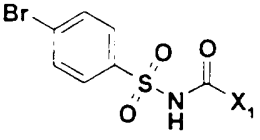
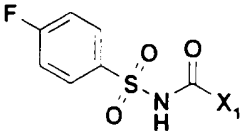
X_1 indicate point of attachment for the R-group.

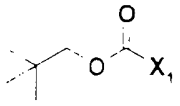
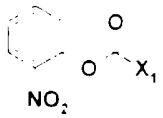
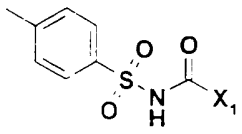
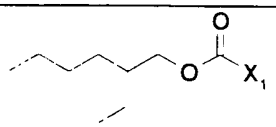
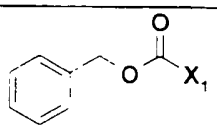
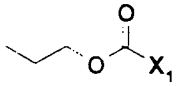
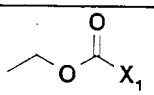
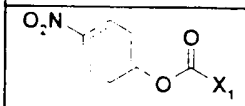
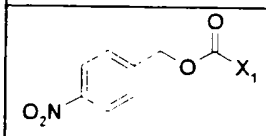
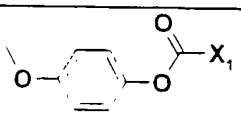
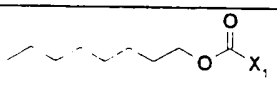
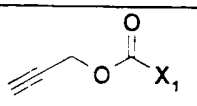
The percentage means the area of the peak of the HPLC at 220 nm.

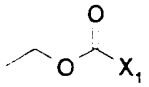
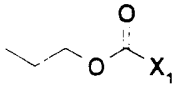
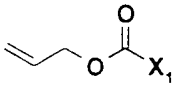
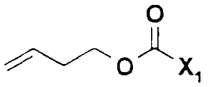
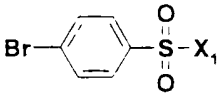
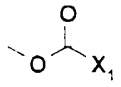
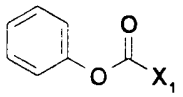
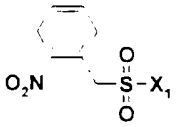
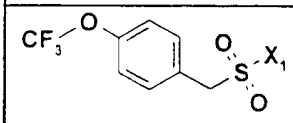
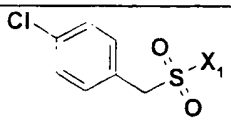
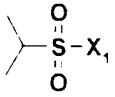
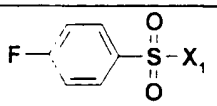
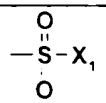
5

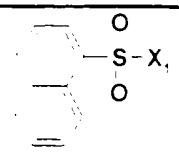
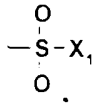
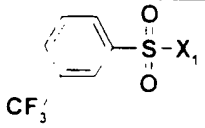
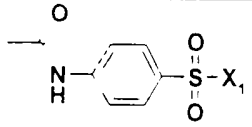
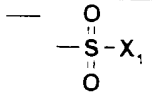
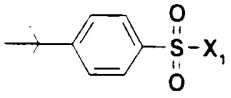
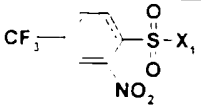
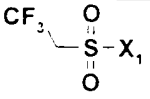
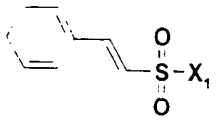
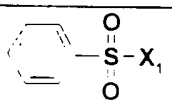
R-group	Formula	Mw	LC/MS
	C13H15N3O8S	373.34	No hit
	C13H17N3O6S	343.36	No hit
	C11H13N3O6S	315.31	No hit
	C15H12N4O8S	408.35	407 (M-H,44%)
	C16H15N3O7S	393.38	No hit
	C17H15N3O7S	405.39	No hit
	C12H15N3O6S	329.33	No hit
	C15H12BrN3O6S	442.25	442 (M-H, 50%)
	C21H25N3O6S	447.51	446 (M-H,92%)

	C15H12N4O8S	408.35	407 (M-H,48%)
	C19H15N3O6S	413.41	412 (M-H,49%)
	C21H17N3O6S	439.45	438 (M-H,81%)
	C17H11F6N3O6S	499.35	498 (M-H,83%)
	C16H12F3N3O6S	431.35	No hit
	C16H12F3N3O6S	431.35	430 (M-H,48%)
	C12H15N3O6S	329.33	328 (M-H,94%)
	C15H19N3O6S	369.40	368 (M-H,85%)
	C16H15N3O7S	393.38	No hit
	C16H15N3O6S	377.38	376 (M-H,86%)

	C17H17N3O8S	423.40	422 (M-H,39%)
	C19H23N3O6S	421.48	420 (M-H,29%)
	C15H13N3O6S	363.35	362 (M-H,26%)
	C15H12N4O8S	408.35	407 (M-H,44%)
	C18H19N3O9S	453.43	452 (M-H, 34%)
	C15H13N3O8S2	427.41	426 (M-H,62%)
	C16H15N3O8S2	441.44	440 (M-H,89%)
	C15H12ClN3O8S2	461.86	460 (M-H,41%)
	C15H11BrN2O7S	443.23	442 (M-H,71%)
	C15H11FN2O7S	382.33	381 (M-H,82%)

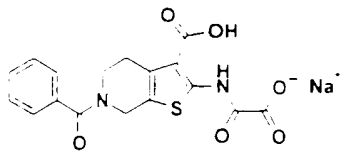
	C14H18N2O7S	358.37	357 (M-H,70%)
	C15H11N3O9S	409.33	408 (M-H,87%)
	C16H15N3O8S2	441.44	No Hit
	C17H24N2O7S	400.45	399 (M-H,68%)
	C16H14N2O7S	378.36	377 (M-H,63%)
	C12H14N2O7S	330.32	329 (M-H,54%)
	C12H14N2O7S	330.32	329 (M-H,76%)
	C15H11N3O9S	409.33	408 (M-H,82%)
	C16H13N3O9S	423.36	422 (M-H,63%)
	C16H14N2O8S	394.36	393 (M-H,78%)
	C17H24N2O7S	400.45	399 (M-H,78%)
	C12H10N2O7S	326.29	325 (M-H,92%)

	C11H12N2O7S	316.29	315 (M-H,70%)
	C13H16N2O7S	344.35	343 (M-H,86%)
	C12H12N2O7S	328.30	327 (M-H,73%)
	C13H14N2O7S	342.33	341 (M-H,74%)
	C14H11BrN2O7S2	463.28	362 (M-H,45%)
	C10H10N2O7S	302.26	301 (M-H,72%)
	C15H12N2O7S	364.34	363 (M-H,82%)
	C15H13N3O9S2	443.41	442 (M-H,94%)
	C15H11F3N2O8S2	468.39	467 (M-H,62%)
	C14H11ClN2O7S2	418.83	417 (M-H,31%)
	C11H14N2O7S2	350.37	349 (M-H,89%)
	C14H11FN2O7S2	402.38	401 (M-H,34%)
	C9H10N2O7S2	322.32	321 (M-H,50%)

	C18H14N2O7S2	434.45	433 (M-H,42%)
	C10H12N2O7S2	336.34	335 (M-H,46%)
	C15H11F3N2O7S2	452.39	451 (M-H,82%)
	C16H15N3O8S2	441.44	440 (M-H,42%)
	C11H14N2O7S2	350.37	349 (M-H,57%)
	C18H20N2O7S2	440.50	439(M-H,42%)
	C15H10F3N3O9S2	497.38	496 (M-H,68%)
	C10H9F3N2O7S2	390.32	389 (M-H,92%)
	C16H14N2O7S2	410.43	409 (M-H,46%)
	C14H12N2O7S2	384.39	383 (M-H,42%)

5

EXAMPLE 81



6-Benzoyl-2-(oxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid, mono sodium salt;

A mixture of N-benzoyl-4-piperidone (20.0 g, 0.1 mol), ethyl cyanoacetate (10.9 ml, 0.1 mol), ammonium acetate (2.0 g) and acetic acid (6 ml) in benzene (100 ml) was heated at reflux temperature in a 3-nacked reaction flask equipped with a Dean-Stark water trap for 1 h. The cooled reaction mixture was diluted with ethyl acetate (100 ml) washed with water (3 x 100 ml), saturated aqueous sodium chloride (80 ml), dried ($MgSO_4$) filtered and evaporated in vacuo affording quantitative yield of (1-benzoyl-piperidin-4-ylidene)-cyano-acetic acid ethyl ester as a slowly crystallising oil.

A mixture of the above benzoyl-piperidin-4-ylidene (10.0 g, 0.034 mol), sulphur (1.13 g, 0.035 mol), morpholin (6.5 ml) in ethanol (35 ml) was heated at 50 °C for 2 h and stirred at room temperature over night. The precipitate was filtered off and washed with 96 % ethanol (3 x 50 ml), diethyl ether (3 x 50 ml) and dried in vacuo which afforded 9.27 g (84 %) of 2-amino-6-benzoyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid ethyl ester as a solid.

To a stirred solution of the above 4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid ethyl ester (5.0 g, 0.015 mol), triethylamine (4.21 ml, 0.03 mol) in dry tetrahydrofuran (30 ml) at 0 °C was added dropwise a solution of ethyl oxalyl chloride (1.9 ml, 0.017 mol) in dry tetrahydrofuran (20 ml). The resulting reaction mixture was stirred at room temperature for 18 h, pored into ice water (300 ml) and extracted with ethyl acetate (3 x 100 ml). The combined organic extracts were washed with saturated aqueous sodium chloride (100 ml), dried ($MgSO_4$) filtered and evaporated in vacuo affording 4.2 g (84 %) of 6-benzoyl-2-(ethoxyoxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid ethyl ester as a crystallising oil

To a solution of the above thieno[2,3-c]pyridine-3-carboxylic acid ethyl ester (4.2 g, 9.76 mmol) in ethanol (100 ml) was added a solution of sodium hydroxide (0.9 g, 21.46 mmol) in water (100 ml). The resulting reaction mixture was stirred at room temperature for 18 h. The volatiles were evaporated in vacuo and the residue dissolved in water (100 ml) and washed with ethyl acetate (2 x 100 ml). To the aqueous phase was added concentrated hydrochloric acid to pH = 1 and the precipitate was filtered off and washed with water (2 x 50 ml), diethyl ether (2 x 30 ml) and dried in vacuo at 50 °C affording 2.9 g (79 %) of the title compound as a solid.

M.p.: Amorph:

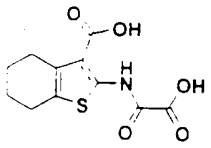
Calculated for $C_{17}H_{13}N_2O_6S_1Na_1$, 1 x H_2O ;

C, 49.28 %; H, 3.65 %; N, 6.76%. Found:

C, 49.31 %; H, 3.86 %; N, 6.53%.

By a similar procedure as described in Example 81 the following compounds have been prepared.

EXAMPLE 82



2-(Oxalyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid;

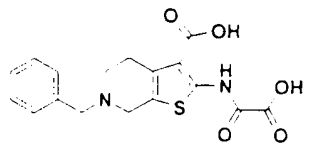
M.p.: 230 - 231 °C:

Calculated for $C_{11}H_{11}NO_5S$;

C, 49.07 %; H, 4.12 %; N, 5.20%. Found:

C, 49.87 %; H, 4.37 %; N, 5.06%.

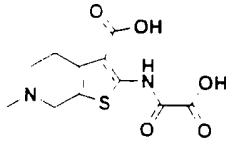
EXAMPLE 83



6-Benzyl-2-(oxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid;

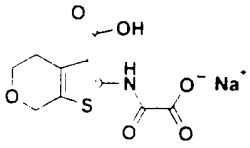
Calculated for $C_{17}H_{16}N_2O_5S$, 1.75 H_2O;
C, 52.10 %; H, 5.01 %; N, 7.15 %. Found:
C, 52.11 %; H, 4.81 %; N, 7.01 %.

EXAMPLE 84



6-Methyl-2-(oxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid;
M.p.: > 250 °C
Calculated for $C_{11}H_{12}N_2O_5S$, 0.6 H_2O;
C, 44.77 %; H, 4 51 %; N, 9.49 %. Found:
C, 44.54 %; H, 4.17 %; N, 9.21 %.

EXAMPLE 85



2-(Oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid, mono sodium salt;

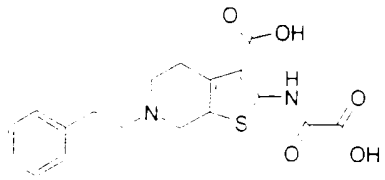
M.p.: > 250 °C

Calculated for $C_{10}H_8N_1O_6SNa$, 0.75 x H_2O;

C. 39.16 %; H. 3.12 %. N. 4.57 %. Found.

C. 39.29 %. H. 3.67 %. N. 4.41 %.

EXAMPLE 86



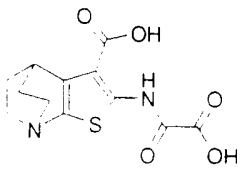
2-(Oxalyl-amino)-6-phenethyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid;

Calculated for $C_{18}H_{18}N_2O_5S$, 1 x H_2O;

C, 55.09 %; H, 5.14 %; N, 7.14 %. Found:

C, 55.47 %; H, 5.04 %; N, 7.07 %.

EXAMPLE 87



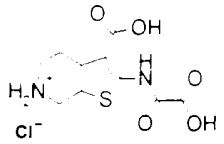
2-(Oxalyl-amino)-4,5,6,7-tetrahydro-4,7-ethano-thieno[2,3-b]pyridine-3-carboxylic acid;

Calculated for $C_{12}H_{12}N_2O_5S$, 0.75 x H_2O;

C, 46.52 %; H, 4.39 %; N, 9.04 %. Found:

C, 46.48 %; H, 4.79 %; N, 8.87 %.

EXAMPLE 88



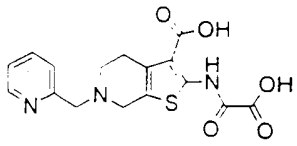
2-(Oxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid, hydrochloride;

4-Oxo-1-piperidine carboxylic acid *tert*-butyl ester was used as starting material. The Boc-group was removed using 25 % trifluoroacetic acid in dichloromethane.

M.p.: > 250 °C

Calculated for $C_{10}H_{10}N_2O_5S$, 1 HCl, 0.5 x H_2O;
C, 38.35 %; H, 4.34 %; N, 8.64 %. Found:
C, 38.04 %; H, 3.83 %; N, 8.87 %.

EXAMPLE 89



2-(Oxalyl-amino)-6-pyridin-2-ylmethyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid;

To a mixture of 2-(ethoxyoxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid ethyl ester trifluoroacetic acid salt (1.5 g, 3.40 mmol), potassium carbonate (2.4 g, 17.1 mmol), potassium iodine (100 mg) in acetone (40 ml) was added 2-picolyl chloride hydrochloride (0.61 g, 3.7 mmol). The resulting mixture was stirred at reflux temperature for 18 h, filtered and evaporated in vacuo. The residue was trituated with diethyl ether and the solid was filtered off and purified on silicagel (300 ml) using a mixture of ethyl ace-

tate/ethanol/triethyl amine (3:1:0.4) as eluent. Pure fractions were collected and the eluent evaporated in vacuo affording 650 mg (39 %) of 2-(ethoxyoxalyl-amino)-6-pyridin-2-ylmethyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid triethyl ammonium salt as a solid.

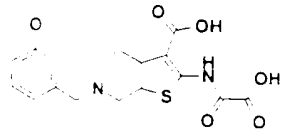
To a solution of the above triethyl ammonium salt (650 mg, 1.40 mmol) in ethanol (15 ml) was added 1 N aqueous sodium hydroxide (4.1 ml, 4.1 mmol) followed by water (15 ml). The resulting reaction mixture was stirred at room temperature for 18 h. The volatiles were evaporated in vacuo and the residue dissolved in water (20 ml) and washed with diethyl ether (2 x 10 ml). To the aqueous phase was added 1N hydrochloric acid to pH = 1 and the aqueous phase was evaporated in vacuo. The residue was suspended in a mixture of 2-propanol/water (1:1, 40 ml), stirred for 1 h., the solid filtered off and washed with 2-propanol (2 x 15 ml) and dried in vacuo at 50 °C affording 181 mg (38 %) of crude title compound. The crude product (181 mg) was dissolved in a mixture of water (10 ml) and5 N sodium hydroxide (10 ml) and washed with diethyl ether (2 x 10 ml). The aqueous phase was acidified to pH = 3 with 1 N hydrochloric acid and the precipitate filtered off and washed with water (3 x 20 ml), dried in vacuo at 50 °C for 18 h which afforded 51 mg (11%) of the title compound as a solid.

M.p.: 238 - 244 °C

Calculated for $C_{16}H_{15}N_3O_5S$, 2.5 x H_2O;
C, 47.29 %; H, 4.96 %; N, 10.34 %. Found:
C, 47.43 %; H, 4.84 %; N, 10.00 %.

By a similar procedure as described in Example 89 the following compounds were prepared.

EXAMPLE 90

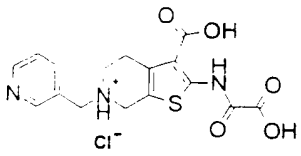


6-(3-Methoxy-benzyl)-2-(oxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid;

M.p.: 233 - 237 °C

Calculated for $C_{18}H_{18}N_2O_6S$, 1 x H_2O;
C, 52.93 %; H, 4.94 %; N, 6.86 %. Found:
C, 52.79 %; H, 4.99 %; N, 6.42 %.

EXAMPLE 91

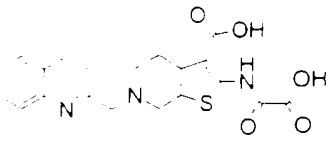


2-(Oxalyl-amino)-6-pyridin-3-ylmethyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid, hydrochloride;

M.p.: 234 - 238 °C

Calculated for $C_{16}H_{15}N_3O_5S$, 1 x HCl, 0.5 x H_2O;
C, 47.24 %; H, 4.21 %; N, 10.33 %. Found:
C, 47.35 %; H, 4.10 %; N, 10.35 %.

EXAMPLE 92

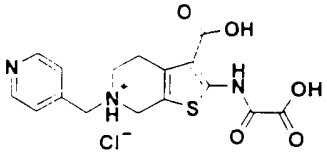


2-(Oxalyl-amino)-6-quinolin-2-ylmethyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid;

M.p.: > 250 °C

Calculated for $C_{20}H_{17}N_3O_5S$, 1 x H_2O;
C, 55.95 %; H, 4.22 %; N, 9.61 %. Found:
C, 55.94 %; H, 4.46 %; N, 9.78 %.

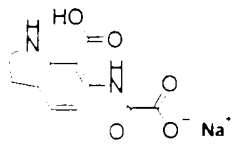
EXAMPLE 93



2-(Oxalyl-amino)-6-pyridin-4-ylmethyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid, hydrochloride;

M.p.. 230 - 235 °C
Calculated for $C_{16}H_{15}N_3O_5S$, 1 x HCl, 1 x H_2O;
C, 46.21 %; H, 4 36 %; N, 10.10 %. Found:
C, 45.82 %; H, 4.42 %; N, 10.02 %.

EXAMPLE 94



6-(Oxalyl-amino)-1H-indole-7-carboxylic acid, mono sodium salt;

To a stirred solution of 6-amino-1H-indole-7-carboxylic acid ethyl ester (1.5 g, 7.3 mmol, prepared as described in *J. Org. Chem.* **61**, 1155-1158 (1996)), triethylamine (1.55 ml, 11.0 mmol) in dry tetrahydrofuran (100 ml) at 0 °C was added dropwise a solution of ethyl oxalyl chloride (980 ul, 88.0 mmol) in dry tetrahydrofuran (10 ml). The resulting reaction mixture was stirred at room temperature for 2 h. pored into ice water (300 ml) and the precipitate filtered off and dried in vacuo at 50 °C affording 2.25 g (100 %) of 6-(ethoxyoxalyl-amino)-1H-indole-7-carboxylic acid ethyl ester as an oil.

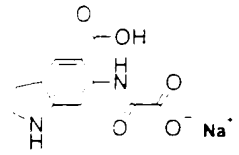
To a solution of the above 1H-indole-7-carboxylic acid ethyl ester (2.0 g, 6.60 mmol) in ethanol (30 ml) was added 1N aqueous sodium hydroxide (16.4 ml, 16.4 mmol) in water (30 ml). The resulting reaction mixture was stirred at room temperature for 18 h. The volatiles were evaporated in vacuo and to the residual aqueous phase was added 1N hydrochloric acid to pH = 1. The precipitate was filtered off and washed with water (2 x 50 ml), diethyl ether (2 x 30 ml) and dried in vacuo at 50 °C affording 1.34 g (82 %) of the title compound as a solid.

M.p.: > 250 °C

Calculated for $C_{11}H_7N_2O_5Na$, 1.5 x H_2O;
C, 44.46 %; H, 3.39 %; N, 9.43 %. Found:
C, 44.31 %; H, 3.34 %; N, 9.00 %.

By a similar procedure as described in Example 94 the following compound was prepared.

EXAMPLE 95



6-(Oxalyl-amino)-1H-indole-5-carboxylic acid, mono sodium salt:

6-amino-1H-indole-5-carboxylic acid ethyl ester was prepared as described in *J. Org. Chem.* **61**, 1155-1158 (1996)).

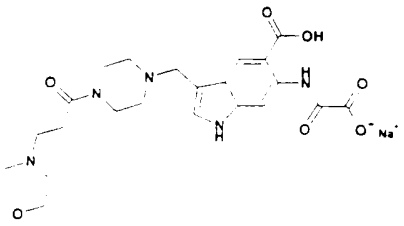
M.p.: > 250 °C

Calculated for $C_{11}H_7N_2O_5Na$, 1.5 x H_2O;

C, 44.46 %; H, 3.39 %; N, 9.43 %. Found:

C, 44.44 %; H, 3.68 %; N, 9.00 %.

EXAMPLE 96



3-[4-(3-Morpholin-4-yl-propionyl)-piperazin-1-ylmethyl]-6-(oxalyl-amino)-1H-indole-5-carboxylic acid, mono sodium salt:

To a ice cooled solution of 37 % aqueous formaldehyde (2.7 g, 33.0 mmol) in acetic acid (8 ml) was added dropwise a solution of piperazine-1-carboxylic acid tert-butyl ester (2.7 g, 15 mmol). After stirring for 15 min. a solution of 6-(ethoxyoxalyl-amino)-1H-indole-5-carboxylic acid (4.0 g, 13.0 mmol) in a mixture of acetic acid (80 ml) and tetrahydrofuran (80 ml) was added and the resulting reaction mixture was stirred for 18 h. at room temperature. The volatiles were evaporated in vacuo and to the residue was added water (100 ml). The aqueous phase was extracted with ethyl acetate (2 x 100 ml), the combined organic extracts were

washed with water (2 x 100 ml), saturated aqueous ammonium chloride (1 x 80 ml), dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was trituated with diethyl ether (50 ml) and the precipitate was filtered off and washed with diethyl ether, dried in vacuo at 50°C which afforded 3.4 g (51 %) of 3-(4-*tert*-butoxycarbonyl-piperazin-1-ylmethyl)-6-(ethoxyoxalyl-amino)-1H-indole-5-carboxylic acid ethyl ester as a solid.

To a solution of the above 6-(ethoxyoxalyl-amino)-1H-indole-5-carboxylic acid ethyl ester in dichloromethane (20 ml) was added trifluoroacetic acid (20 ml) at room temperature. The resulting mixture was stirred for 1 h., the volatiles were evaporated in vacuo and to the residue was added water (50 ml) and the resulting mixture was stirred for ½ h. The precipitate was filtered off and washed with water (50 ml), diethyl ether (50 ml) and in vacuo at 50 °C which afforded 3.6 g (100 %) of 6-(ethoxyoxalyl-amino)-3-piperazin-1-ylmethyl-1H-indole-5-carboxylic acid ethyl ester trifluoroacetic acid salt as a solid.

To a ice cooled mixture of the above piperazin (3.0 g, 5.81 mmol) in dichloromethane (100 ml) and triethylamine (2.5 ml) was added dropwise a mixture of chloropropionyl chloride (0.6 ml, 6.39 mmol) in dichloromethane (10 ml). The resulting mixture was stirred for 1 h. at room temperature, washed with water (50 ml), dried ($MgSO_4$), filtered and evaporated in vacuo affording 1.8 g (68 %) of 3-(4-acryloyl-piperazin-1-ylmethyl)-6-(ethoxyoxalyl-amino)-1H-indole-5-carboxylic acid ethyl ester
as a oil.

To a solution of the above acryloyl-piperazin (0.5 g, 1.1 mmol) in ethanol (50 ml) was added morpholin (0.24 g, 2.74 mmol). The resulting mixture was stirred at reflux temperature for 18 h. and the volatiles were evaporated in vacuo. The residue was dissolved in water (50 ml), pH was adjusted to 2 with 1N hydrochloric acid and washed with ethyl acetate (2 x 50 ml). The aqueous phase was neutralised with 1N sodium hydroxide, the precipitate was filtered off, washed with water and dried in vacuo at 50 °C for 3h which afforded 0.3 g (50 %) of 6-(ethoxyoxalyl-amino)-3-[4-(3-morpholin-4-yl-propionyl)-piperazin-1-ylmethyl]-1H-indole-5-carboxylic acid ethyl ester as a solid.

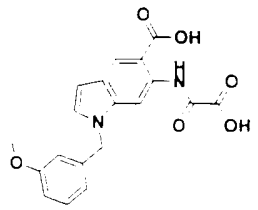
To a solution of the above 1H-indole-5-carboxylic acid ethyl ester (0.2 g, 0.37 mmol) in ethanol (5 ml) was added sodium hydroxide (45 mg, 1.10 mmol) in water (15 ml). The resulting reaction mixture was stirred at room temperature for 18 h, pH adjusted to 1 by addition of 1N

hydrochloric acid. The aqueous phase was washed with ethyl acetate (2 x 25 ml) and pH adjusted to 5 by addition of 1N sodium hydroxide, followed by addition of dichloromethane (25 ml). The precipitate was filtered off and washed with water (50 ml) and dried in vacuo at 50 °C affording 30 mg (17 %) of the title compound as a solid.

M.p.: > 250 °C

LC-MS (E^+) M/Z 488

EXAMPLE 97



1-(3-Methoxy-benzyl)-6-(oxalyl-amino)-1H-indole-5-carboxylic acid:

To a solution of 6-amino-1H-indole-5-carboxylic acid ethyl ester (1.0 g, 3.3o mmol; prepared as described in *J. Org. Chem.* **61**, 1155-1158 (1996)) in dry N,N-dimethylformamide (40 ml) was added sodium hydride (0.28 g, 7.3 mmol; 60 % in mineral oil). The reaction mixture was stirred for 1.5 h and a solution of 3-methoxybenzylchloride (0.5 ml, 3.6 mmol) in dry N,N-dimethylformamide (2.5 ml) was added dropwise. The resulting reaction mixture was stirred for 1.5 h, poured into water (300 ml) and washed with diethyl ether (3 x 100 ml). Undissolved matter was filtered off and the aqueous phase was acidified to pH = 4 by addition of 1N hydrochloric acid. The precipitate was filtered off and washed with water, dried in vacuo at 50 °C affording 400 mg (29 %) of 6-(ethoxyoxalyl-amino)-1-(3-methoxy-benzyl)-1H-indole-5-carboxylic acid ethyl ester as a solid.

To a solution of the above 1H-indole-5-carboxylic acid ethyl ester (0.3 g, 0.7 mmol) in ethanol (10 ml) was added 1N sodium hydroxide (2.1 ml, 2.1 mmol) and water (10 ml). The resulting reaction mixture was stirred at room temperature for 18 h. The volatiles were evaporated in vacuo, pH adjusted to 2 by addition of 1N hydrochloric acid, the precipitate filtered

off and washed with water, dried in vacuo at 50 °C affording 230 mg (89 %) of the title compound as a solid.

M p.: 222 - 226 °C

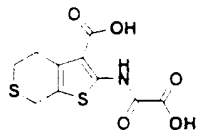
Calculated for $C_{19}H_{16}N_2O_6$, 0.4 x H_2O:

C, 60.77 %; H, 4.51 %; N, 7.46 %. Found:

C, 60.96 %; H, 4.44 %; N, 7.28 %.

By a similar procedure as described in Example 81 the following compound was prepared.

EXAMPLE 98



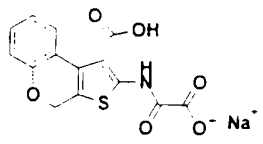
2-(Oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]thiopyran-3-carboxylic acid;

Calculated for $C_{10}H_9NO_5S_2$;

C, 41.80 %; H, 3.16 %; N, 4.88 %. Found:

C, 41.97 %; H, 3.20 %; N, 4.69 %.

EXAMPLE 99



2-(Oxalyl-amino)-9H-thieno[2,3-c]chromen-3-carboxylic acid, mono sodium salt

To a solution of 4-cromanone (20 g, 0.14 mol), ethyl cyanoacetate (16.8 g, 0.15 mol) and ammonium acetate (11.4 g, 0.15 mol) in benzene (500 ml) was added acetic acid (5 ml), the

resulting reaction mixture was heated at reflux temperature for 18 h and the formed water was collected in a Dean-Stark water trap. An additional portion of ammonium acetate (10 g 0.13 mol) was added and heating at reflux temperature was continued for an additional 8 h. The volatiles were evaporated in vacuo, to the residue was added water (500 ml) and the aqueous phase was extracted with ethyl acetate (2 x 200 ml). The combined organic extracts were washed with water (2 x 100 ml), saturated aqueous sodium chloride (100 ml), dried ($MgSO_4$), filtered and evaporated in vacuo afforded 28 g of a 1:1 mixture of unchanged starting material and chroman-4-ylidene-cyano-acetic acid ethyl ester as an oil.

To a solution of the crude product in ethanol (250 ml) was added sulphur (2.5 g, 0.08 mol) and morpholin (15 ml). The resulting reaction mixture was stirred at 50 °C for 4 h cooled to room temperature and filtered. The volatiles were evaporated in vacuo affording 30 g of crude product.

The product was divided into two portions which was semi purified on silica gel (900 ml) using a mixture of ethyl acetate/heptane (1:3). Semi pure fractions were collected and the solvent evaporated in vacuo affording a crude oil which was dissolved in diethyl ether (80 ml) and crystallised by addition of heptane (125 ml). The precipitated was filtered off, washed with heptane and dried in vacuo at 50 °C for 18 h affording 8.9 g (24 %) of 2-amino-9H-thieno[2,3-c]chromen-3-carboxylic acid ethyl ester as a solid.

To a stirred solution of the above 2-amino-6H-thieno[2,3-c]chromen-3-carboxylic acid ethyl ester (2.9 g, 10.53 mmol), triethylamine (3 ml) in dry tetrahydrofuran (100 ml) at 0 °C was added dropwise a solution of ethyl oxalyl chloride (1.6 g, 11.6 mmol) in dry tetrahydrofuran (20 ml). The resulting reaction mixture was stirred at room temperature for 1.5 h. pored into ice water (200 ml) and the precipitate filtered off and dried in vacuo at 50 °C affording 2.6 g (66 %) of 2-(ethoxyoxalyl-amino)-9H-thieno[2,3-c]chromen-3-carboxylic acid ethyl ester as a solid.

To a solution of the above ethyl ester (1.5 g, 4.0 mmol) in ethanol (25 ml) was added sodium hydroxide (480 mg, 12 mmol) and water (50 ml). The resulting reaction mixture was stirred at room temperature for 42 h. Water (100 ml) was added and the mixture was washed with diethyl ether (100 ml). pH of the aqueous phase was adjusted to 1 by addition of concentrated hydrochloric acid, the precipitate was filtered off, washed with water and dried in vacuo at 50 °C for 6 h affording 0.6 g (47 %) of the title compound as a solid.

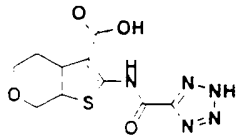
M.p.: 227 - 228 °C

Calculated for $C_{14}H_9NO_6SNa$, 0.5 H_2O:

C, 48.01 %; H, 2.59 %; N, 4.00 %. Found:

C, 48.39 %; H, 2.93 %; N, 3.93 %.

EXAMPLE 100



2-((2-H-Tetrazol-5-carbonyl)amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid:

To a mixture of N,N-dimethylformamide (1.6 ml) and acetonitrile (5 ml) cooled to -20 °C was added dropwise a mixture of oxalyl chloride (0.8 g, 6.31 mmol) in acetonitrile (1 ml). The resulting mixture was stirred for 15 min. and tetrazole-5-carboxylic acid dipotassium salt (1 g, 5.25 mmol, prepared as described in *J. Med. Chem.* **29**, 538-549 (1986)) was added and the resulting mixture was stirred for an additional 20 min. To the mixture was added dropwise a solution of 2-amino-4,5-dihydro-7H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (1.3 g, 5.25 mmol), pyridine (2.2 ml) and acetonitrile (2.5 ml) during 10 min. The reaction mixture was allowed to reach room temperature where after it was heated at reflux temperature for 0.5 h. The cooled reaction mixture was pored into water (100 ml) and pH was adjusted to 1 by addition of concentrated hydrochloric acid. The precipitate was filtered off, washed with heptane and dried in vacuo at 50 °C for 18 h affording 1.3 g (70 %) of 2-((1H-tetrazole-5-carbonyl)-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

The above *tert*-butyl ester (0.6 g, 1.71 mmol) was dissolved in dichloromethane (5 ml) and trifluoroacetic acid (5 ml) was added. The resulting mixture was stirred for 40 min. at room temperature. The volatiles were evaporated in vacuo and to the residue was added diethyl ether (50 ml), water (25 ml) and 1 N sodium hydroxide (2 ml). The phases were separated and the aqueous phase was washed with diethyl ether (50 ml) and pH was adjusted to 1 by addition of concentrated hydrochloric acid. The precipitate was filtered off, washed with water (25 ml) and dried in vacuo at 50 °C for 18 h which afforded 190 mg (38 %) of the title compound as a solid.

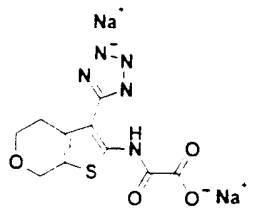
M.p.: > 250 °C

Calculated for $C_{10}H_5N_5O_4S$, 0.25 x H_2O,

C, 40.07 %; H, 3.19 %; N, 23.36 % Found:

C, 40.39 %; H, 3.18 %; N, 22.92 %

EXAMPLE 101



N-(3-(2H-Tetrazol-5-yl)-4,7-dihydro-5H-thieno[2,3-c]pyran-2-yl)oxalamic acid, di sodium salt

2-Amino-4,5-dihydro-7H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (26 g, 0.114 mol) was dissolved in formamide (200 ml) and the resulting mixture was heated at reflux temperature for 1.5 h. After cooling to room temperature the precipitate was filtered off, washed with water (2 x 80 ml) and dried in vacuo at 50 °C for 18 h which afforded 10.0 g (42 %) of 5,6-dihydro-8H-pyrano[4',3':4,5]thieno[2,3-d]pyrimidin-4-one as a solid.

To phosphorus oxychloride (70 ml) was added the above pyrimidin-4-one (7.0 g, 0.04 mol) and N,N-dimethylaniline (0.2 ml). The resulting mixture was heated at reflux temperature for 2 h, cooled and pored onto ice water (700 ml). The precipitate was filtered off, suspended in a mixture of ethyl acetate (400 ml) and water (250 ml) and stirred for 15 min. The aqueous phase was separated off and the organic phase was washed with saturated aqueous sodium chloride (100 ml), dried ($MgSO_4$), filtered and evaporated in vacuo which afforded 5.2 g (68 %) of 4-chloro-5,6-dihydro-8H-pyrano[4',3':4,5]thieno[2,3-d]pyrimidine as a solid.

To a warm solution of the above thieno-pyrimidine (4.5 g, 0.02 mol) in ethanol (40 ml) was added dropwise a solution of hydrazine hydrate (10.0 ml) in ethanol (20 ml). The resulting solution was heated at reflux temperature for 2 h, cooled to room temperature, the precipitate filtered off, washed with ethanol (20 ml) and dried in vacuo at 50 °C for 1.5 h affording

3.2 g (73 %) of 5,6-dihydro-8H-pyrano[4',3':4,5]thieno[2,3-d]pyrimidin-4-yl hydrazine as a solid.

To a solution of the above hydrazine (3.0 g, 0.014 mol) in 50 % aqueous acetic acid (100 ml) cooled in a ice bath was added dropwise a solution of sodium nitrite (1.0 g, 0.015 mol) in water (10 ml). The reaction mixture was stirred for 2 h, the precipitate filtered off, washed with water (25 ml) and dried in vacuo at 50 °C for 1 h affording 3.0 g (95 %) of 10,11-dihydro-8H-pyrano[4',3':4,5]thieno[3,2-e]tetrazolo[5,1-c]pyrimidine as a solid.

To a solution of the above tetrazol (2.5 g, 0.011 mol) in dioxane (30 ml) was added dropwise 1 N sodium hydroxide (25 ml). The reaction mixture was stirred for 3 h, pored into ice cooled water (100 ml) and pH was adjusted to 4 by addition of acetic acid. The precipitate was filtered off, washed with water (25 ml) and dried in vacuo at 50 °C for 18 h affording 2.2 g (82 %) of N-(3-(2H-tetrazol-5-yl)-4,7-dihydro-5H-thieno[2,3-c]pyran-2-yl)formamide as a solid.

The above formamide (0.6 g, 2.7 mmol) was dissolved in dry tetrahydrofuran (50 ml) and triethylamine (1 ml) was added. To the resulting mixture cooled in a ice bath was added dropwise a solution of ethyl oxalylchloride (0.4 g, 2.96 mmol) in dry tetrahydrofuran (5 ml). The resulting reaction mixture was stirred for 2 h at room temperature, the volatiles were evaporated in vacuo. To the residue was added water (50 ml), diethyl ether (50 ml) and 1 N hydrochloric acid to pH = 2 and a small precipitate was filtered off. The organic phase was separated, dried (Na_2SO_4), filtered and evaporated in vacuo. The residue (0.4 g) was suspended in dichloromethane (20 ml) and stirred for 1 h, the solid matter was filtered off and dried in vacuo at 50 °C affording 0.16 g (18 %) of N-(3-(2H-tetrazol-5-yl)-4,7-dihydro-5H-thieno[2,3-c]pyran-2-yl)oxalamic acid ethyl ester as a solid.

To a solution of the above oxalamic acid ethyl ester (0.16 g, 0.49 mmol) in ethanol (15 ml) was added 1 N sodium hydroxide (1.0 ml, 1.01 mmol). The resulting reaction mixture was stirred at room temperature for 2 h. The precipitate was filtered off and washed with ethanol (10 ml), dried in vacuo at 50 °C for 48 h affording 140 mg (83 %) of the title compound as a solid.

M.p.: > 250 °C

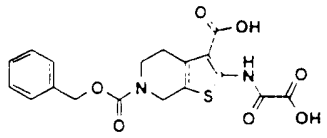
Calculated for $C_{10}H_9N_5O_4SNa_2$, 3 x H_2O;

C, 30.54 %; H, 3.33 %; N, 17.81 %. Found:

C, 30.70 %; H, 3.35 %; N, 17.49 %.

By a similar procedure as described in Example 81 the following compounds were prepared.

EXAMPLE 102



2-(Oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyridine-3,6-dicarboxylic acid 6-benzyl ester

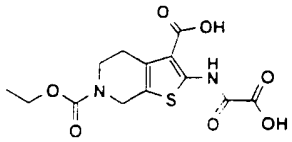
M.p.: >250 °C

Calculated for $C_{18}H_{16}N_2O_7S$;

C, 53.46 %; H, 3.99 %; N, 6.93 %. Found:

C, 53.44 %; H, 4.15 %; N, 6.69 %.

EXAMPLE 103



2-(Oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyridine-3,6-dicarboxylic acid 6-ethyl ester

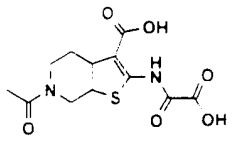
M.p.: 245 - 247 °C

Calculated for $C_{13}H_{14}N_2O_7S$;

C, 45.61 %; H, 4.12 %; N, 8.18 %. Found:

C, 45.71 %; H, 4.31 %; N, 7.86 %.

EXAMPLE 104



6-Acetyl-2-(oxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid

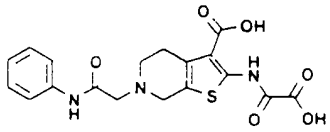
M.p.: 242 - 244 °C

Calculated for $C_{12}H_{12}N_2O_6S$, 0.25 x H_2O;

C, 45.50 %; H, 3.98 %; N, 8.84 %. Found:

C, 45.64 %; H, 3.97 %; N, 8.51 %.

EXAMPLE 105



2-(Oxalyl-amino)-6-phenylcarbamoylmethyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine-3-carboxylic acid

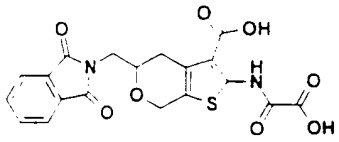
M.p. 244 - 246 °C

Calculated for $C_{18}H_{17}N_3O_6S$, 1 x H_2O;

C, 51.30 %; H, 4.54 %; N, 9.97 %. Found:

C, 51.08 %; H, 4.52 %; N, 9.63 %.

EXAMPLE 106



5-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

To a mixture of benzyloxyacetaldehyde (8.3 g, 0.06 mol) in benzene (80 mL) was added 1-methoxy-3-trimethylsilyloxy-1,3-butadiene (10.6 g, 0.06 mol). The reaction mixture was stirred under nitrogen for 15 min., cooled to 0 °C and a solution of 0.5 M zinc chloride (55 ml 0.03 mol) was added dropwise. The reaction mixture was allowed to warm to room temperature over 16 h and evaporated in vacuo. The resultant oil was diluted with ethyl acetate (100 ml), washed with 1N hydrochloric acid (3 x 50ml), saturated sodium bicarbonate (3 x 50 ml), brine (3 x 50 ml), dried ($MgSO_4$) and evaporated in vacuo. The resulting oil was subjected to flash chromatography using a mixture of ethyl acetate/hexanes (1:2) as eluent. Pure fractions were collected affording after evaporation in vacuo 7.1 g (60 %) of benzyloxymethyl-2,3-dihydro-pyran-4-one as an oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.31 (m, 6H), 5.42 (dd, J = 6,1 Hz, 1H), 4.61 (d, J = 3 Hz, 1H), 4,57 (m, 1H), 3.70 (m, 2H), 2.74 (dd, J = 17 Hz, 14 Hz, 1H), 2.41 (ddd, J = 17 Hz, 2 Hz, 1 Hz, 1H).

The above 2,3-dihydro-pyran-4-one (7.1 g, 0.032 mol) and 10 % palladium on carbon (0.4 g) in ethyl acetate (50 ml) were placed in a Parr bomb shaker and hydrogenated at 30 psi. The reaction mixture was shaken for 2 h, at which time TLC analysis (methanol/dichloromethane 1:9) indicated the reaction was complete. The reaction mixture was filtered through a pad of Celite and the volatiles evaporated in vacuo. The residue was subjected to flash column chromatography using ethyl acetate as eluent. Pure fractions were collected affording after evaporation in vacuo 3.0 g (75 %) of 2-hydroxymethyl-tetrahydro-pyran-4-one as an oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.36 - 4.29 (m, 1H), 3.77 - 3.66 (m, 3H), 3.61 - 3.54 (m, 1H), 2.65 - 2.43 (m, 2H), 2.34 - 2.27 (m, 2H), 2.04 (bs, 1H, CH_2OH).

The above tetrahydro-pyran-4-one (1.90 g, 0.015 mol), *tert*-butyl cyanoacetate (2.7 g, 0.019 mol), sulfur (0.51 g, 0.016 mol) and morpholine (2.55 ml, 0.03 mol) were dissolved in absolute ethanol (20 ml), and heated to 50 °C for 16 h. The reaction mixture was cooled, filtered and the filtrate evaporated in vacuo. The resultant oil was dissolved in ethyl acetate (50 ml), washed with water (2 x 50 ml), brine (2 x 50 m) and dried ($MgSO_4$). The solvent was evaporated in vacuo and the residue was subjected to flash column chromatography using ethyl acetate/hexanes (1:1) as eluent. Pure fractions were collected affording after evaporation in

vacuo 3.7 g (90 %) of 2-amino-5-hydroxymethyl-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.64 (s, 2H), 3.80 - 3.67 (m, 3H), 2.77 - 2.72 (m, 1H), 2.57 - 2.53 (m, 1H), 1.54 (s, 9H).

The above carboxylic acid *tert*-butyl ester (3.0 g, 0.015 mol), phthalimide (2.10 g, 0.014 mol) and triphenylphosphine (3.68 g, 0.014) were dissolved in dry tetrahydrofuran (60 ml) and cooled to 0 °C under a nitrogen atmosphere. Diisopropyl azodicarboxylate (DIAD) (2.71 ml, 0.014 mol) was added dropwise at 0 °C and the solution allowed to stir overnight, slowly warming to room temperature. The volatiles were evaporated in vacuo and the resultant solid dissolved in ethyl acetate (60 ml). The organic phase was washed with brine (2 x 50 ml), dried ($MgSO_4$) and evaporated in vacuo. The residue was subjected to flash column chromatography initially eluted with a mixture of ethyl acetate/hexanes (1:3). Once the product began to elute, the eluent mixture was switched to ethyl acetate/hexanes (1:2). Pure fractions were collected affording after evaporation in vacuo 2.90 g (47 %) of 2-amino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 - 7.85 (m, 2H), 7.83 - 7.71 (m, 2H), 5.94 (bs, 2H), 4.59 (d, J = 14 Hz, 1H), 4.52 (d, J = 14 Hz, 1H), 4.0 - 3.98 (m, 2H), 3.83 - 3.79 (m, 1H), 2.87 (d, J = 17 Hz, 1H), 2.58 (dd, J = 17 Hz, 9 Hz, 1H), 1.50 (s, 9H).

To the above 4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert* butyl ester (0.5 g, 1.2 mmol) dissolved in dichloromethane (5 ml), was added triethylamine (0.33 ml, 2.4 mmol) and imidazol-1-yl-oxo-acetic acid *tert* butyl ester (0.47 g, 2.4 mmol) under nitrogen. The reaction mixture was allowed to stir at room temperature for 18 hours. The volatiles were evaporated in vacuo and the solid residue dissolved in ethyl acetate (20 ml). The organic phase was washed with 1% hydrochloric acid (2 x 10 ml), brine (2 x 10 ml), dried ($MgSO_4$). The organic phase was evaporated in vacuo affording 0.64 g (99 %) of 2-(*tert*-butoxyoxalyl-amino)-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.48 (s, 1H, N*H*CO), 7.88 - 7.86 (m, 2H), 7.74 - 7.72 (m, 2H), 4.78 (d, J = 19 Hz, 1H), 4.65 (d, J = 19 Hz, 1H), 4.07 -3.90 (m, 2H), 3.88 - 3.80 (m, 1H), 2.97 (d, J = 17 Hz, 1H), 2.68 (dd, J = 17 Hz, 9 Hz, 1H), 1.58 (s, 9H), 1.54 (s, 9H).

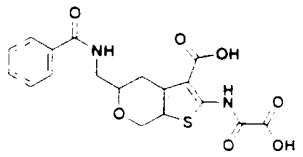
The above di-*tert*-butyl ester (2.8 g, 5.16 mmol) was dissolved in a mixture of trifluoroacetic acid and dichloromethane (1:5) (36 ml). The reaction was stirred at room temperature for 6 hr. The precipitate was filtered off, washed with diethyl ether, dried in vacuo at 50 °C which afforded 1.26 g (57 %) of the title compound as a solid.

M.p.: 245.2 - 245.6 °C.

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.32 (s, 1H, N*H*CO), 7.95 - 7.80 (m, 4H), 4.75 (d, J = 20 Hz, 1H), 4.62 (d, J = 20 Hz, 1H), 3.96 - 3.69 (m, 3H), 3.01 (d, J = 18 Hz, 1H), 2.60 (dd, J = 18 Hz, 9 Hz, 1H).

Calculated for $C_{19}H_{14}N_2O_8S$;
C, 53.02 %; H, 3.28 %; N, 6.51 %. Found:
C, 53.01 %; H, 3.31 %; N, 6.41 %.

EXAMPLE 107



5-(Benzoylamino-methyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid

2-(*tert*-Butoxyoxalyl-amino)-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (0.33 g, 0.60 mmol) was dissolved in a solution of ethanol (2 ml) and dichloromethane (3 ml). Hydrazine (28 µl, 0.9 mmol) was added and the reaction stirred under nitrogen at room temperature for 24 h. TLC analysis indicated that starting material was still present. An additional portion of hydrazine (28 µl, 0.9 mmol) was added and the reaction stirred at room temperature for another 16 h, then at 45 °C for 5 h. The mixture was concentrated in vacuo, redissolved in dichloromethane and the insoluble material filtered off. The filtrate was collected and concentrated in vacuo affording

crude 5-aminomethyl-2-(*tert*-butoxyoxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid, which was carried through to the next step without further purification.

The above crude 5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (0.25 g, 0.60 mmol) was suspended in a mixture of dichloromethane and acetonitrile (1:1, 5 ml). Triethylamine (0.25 ml, 1.8 mmol) was added followed by 1-hydroxy-benzotriazole hydrate (0.10 g, 0.72 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.14 g, 0.72 mmol) as solids. The heterogeneous reaction mixture was allowed to stir at room temperature for 2 days, after which the mixture was homogenous. The solvents were evaporated in vacuo, the residue dissolved in dichloromethane washed twice with 1M hydrochloric acid, then with saturated sodium bicarbonate. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo affording a solid which was purified by flash chromatography using a mixture of ethyl acetate and hexanes (1:1) as eluent. Pure fractions were collected and evaporated in vacuo affording 50 mg (16 % over two steps) of 5-(benzoylamino-methyl)-2-(*tert*-butoxyoxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

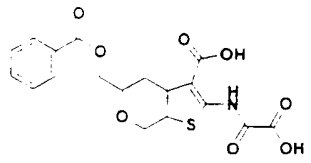
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.46 (s, 1H), 7.81 (d, J = 7 Hz, 2 H), 7.51 -7.42 (m, 3H), 6.72 (bs, 1H), 4.83 (d, J = 17 Hz, 1H), 4.74 (d, J = 17 Hz, 1H), 4.05 - 3.98 (m, 1H), 3.86 - 3.78 (m, 1H), 3.45 - 3.38 (m, 1H), 2.97 (d, J = 19 Hz, 1H), 2.68 (dd, J = 19 Hz, 9 Hz, 1H), 1.61 (s, 9H), 1.58 (s, 9H).

The above benzoylamino-methyl-thieno[2,3-c]pyran (40 mg, 0.078 mmol) was treated with 20 % trifluoroacetic acid in dichloromethane (2 ml) for 4 h. The volatiles were evaporated in vacuo and chased twice with dichloromethane, forming a precipitate which was filtered off and dried yielding 30 mg (95 %) of the title compound as a solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 8.63 (t, J = 4 Hz, 1H), 7.86 (d, J = 7 Hz, 2H), 7.51 - 7.43 (m, 3H), 4.80 (d, J = 17 Hz, 1H), 4.64 (d, J = 17 Hz, 1H), 3.82 (m, 1H), 3.44 (m, 2H), 2.95 (d, J = 18, 1H), 2.52 (dd, J = 18 Hz, 9 Hz, 1H).
LC/MS [M-H]: 403.39.

HPLC (254.4nm): 2.99 s, 84 %.

EXAMPLE 108



5-Benzoyloxymethyl-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid

2-Amino-5-hydroxymethyl-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (0.23 g, 0.87 mmol) benzoic acid (0.10 g, 0.96 mmol) and triethylamine (0.23 ml, 1.7 mmol) were dissolved in dichloromethane (4 ml) and stirred under nitrogen. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.17 g, 0.96 mmol) and 1-hydroxy-benzotriazole hydrate (0.12 g, 0.96 mmol) were added as solids. The reaction mixture was stirred at room temperature for 2 days, after which the solvents were evaporated in vacuo. The crude mixture was dissolved in ethyl acetate and washed with 1N hydrochloric acid, saturated sodium bicarbonate, brine and dried (Na_2SO_4). The solvent was evaporated in vacuo, yielding a yellow solid that was purified by flash chromatography using a mixture of ethyl acetate and hexanes (1:2) as eluent. Pure fractions were collected and evaporated in vacuo affording 0.22 g (70 %) of 2-amino-5-benzoyloxymethyl-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 7 Hz, 2H), 7.55 (t, J = 7 Hz, 1H), 7.42 (t, J = 7 Hz, 2H), 4.64 (s, 2H), 4.44 (d, J = 5 Hz, 2H), 4.03 - 3.97 (m, 1H), 2.88 (d, J = 18 Hz, 1H), 2.64 (dd, J = 17 Hz, 10 Hz, 1H), 1.50 (s, 9H).

LC/MS [M+H]: 390.48

To the above carboxylic acid *tert*-butyl ester (0.18 g, 0.45 mmol) dissolved in dry tetrahydrofuran (5 ml), was added triethylamine (0.18 ml, 1.4 mmol) and imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (0.26 g, 1.4 mmol) under nitrogen. The reaction mixture was stirred at

room temperature for 3 h. The volatiles were evaporated in vacuo and the resultant solid re-constituted in ethyl acetate (10 ml). The organic layer was washed with 1% hydrochloric acid (2 x 10 ml), brine (2 x 10 ml), dried (Na_2SO_4), filtered and the solvent evaporated in vacuo. The resulting oil was purified by flash chromatography using a mixture of ethyl acetate and hexane (1:2) as eluent affording ester 0.20 g (90 %) of 5-benzoyloxymethyl-2-(*tert*-butoxyoxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7 Hz, 2H), 7.56 (t, J = 7 Hz, 1H), 7.44 (t, J = 7 Hz, 2H), 4.85 (d, J = 15 Hz, 1H), 4.77 (d, J = 15 Hz, 1H), 4.49 (d, J = 5 Hz, 2H), 4.03 - 3.99 (m, 1H), 2.99 (d, J = 17 Hz, 1H), 2.72 (dd, J = 17 Hz, 11 Hz, 1H), 1.58 (s, 9H), 1.60 (s, 9H).

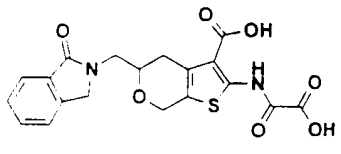
The above di-*tert* butyl ester (0.15 g, 0.29 mmol) was dissolved in a solution of 20 % trifluoroacetic acid in dichloromethane (3 ml). Immediately the solution developed a dark orange color that quickly became red. The reaction was stirred for 1.5 h at room temperature. The volatiles were evaporated in vacuo affording a brown solid which was washed twice with diethyl ether and water and filtered off. The resulting solid was dried in vacuo, yielding 30 mg (25 %) of the title compound as a solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 7.98 (d, J = 7 Hz, 2H), 7.67 (t, J = 7 Hz, 1H), 7.54 (t, J = 7 Hz, 2H), 4.83 (d, J = 15 Hz, 1H), 4.70 (d, J = 15 Hz, 1H), 4.44 (d, J = 5 Hz, 2H), 4.02 - 3.99 (m, 1H), 2.99 (d, J = 16 Hz, 1H), 2.70 (dd, J = 16 Hz, 9 Hz, 1H).

LC/MS [M-H]: 404.05.

HPLC (254.4 nm): 7.16 s, 90 %.

EXAMPLE 109



2-(Oxalyl-amino)-5-(1-oxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid

To a solution of 2-amino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (0.308 g, 0.74 mmol) in absolute ethanol (5 ml) was added hydrazine (47 µl, 1.48 mmol). The reaction was stirred at 80 °C for 4 h and then at room temperature for another 12 h. The precipitate formed was filtered off and the filtrate concentrated in vacuo. To the oily residue was added dichloromethane (15 ml) and the precipitate formed was filtered off. The filtrate was concentrated in vacuo to give 2-amino-5-aminomethyl-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester 0.19 g (90 %) as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (bs, 2H), 4.62 (s, 2H), 3.64 - 3.60 (m, 1H), 2.92 - 2.84 (m, 2H), 2.80 - 2.75 (m, 1H), 2.52 - 2.45 (m, 1H), 1.53 (s, 9H).

LC-MS $[M+H]^+$: 285

Phthalic dicarboxaldehyde (52 mg, 0.36 mmol) was dissolved in a mixture of anhydrous acetonitrile (2 ml) and acetic acid (44 µl, 0.72 mmol). The above 2-amino-5-aminomethyl-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (0.11 g, 0.36 mmol) was added and the reaction stirred for 20 minutes at room temperature. The volatiles were evaporated in vacuo and the residue dissolved in ethyl acetate (25 ml). The organic mixture was washed with saturated sodium bicarbonate (5 ml), 1 % hydrochloric acid (5 ml), brine (5 ml), dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by chromatography using a gradient from 15 % ethyl acetate/dichloromethane to 17 % ethyl acetate/dichloromethane as eluent affording 45 mg (30 %) of 2-amino-5-(1-oxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 7 Hz, 1H), 7.53 (t, J = 7 Hz, 1H), 7.47 - 7.43 (m, 2H), 4.68 (d, J = 17 Hz, 1H), 4.58 - 4.51 (m, 3H), 3.99 (dd, J = 14 Hz, 3 Hz, 1H), 3.93 - 3.89 (m, 1H), 3.66 - 3.61 (m, 1H), 2.88 (d, J = 17 Hz, 1H), 2.55 (dd, J = 17 Hz, 11 Hz, 1H), 1.52 (s, 9H).

To a solution of 2-amino-5-(1-oxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (45 mg, 1.1 mmol) in anhydrous dichloromethane (4 ml) was added imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (73 mg, 3.3 mmol) and triethylamine (17 µl, 1.1 mmol). The reaction was stirred under nitrogen at room temperature for 5 h. The solvent was evaporated in vacuo and the crude material was dissolved in ethyl ace-

tate (20 ml). The organic solution was washed with 0.5 N hydrochloric acid (3 ml), saturated sodium bicarbonate (3 ml), brine (5 ml), dried (Na_2SO_4), filtered and the solvent evaporated in vacuo. The residue was purified by chromatography using dichloromethane (100 %) followed by 17 % ethyl acetate/dichloromethane as eluents affording 54 mg (91 %) of 2-(*tert*-butoxyoxalyl-amino)-5-(1-oxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.50 (s, 1H), 7.84 (d, J = 8 Hz, 1H), 7.53 (t, J = 7 Hz, 1H), 7.47 - 7.43 (m, 2H), 4.81 - 4.65 (m, 3H), 4.53 (d, J = 17 Hz, 1H), 4.01 (dd, J = 14 Hz, 3 Hz, 1H), 3.96 - 3.89 (m, 1H), 3.69 - 3.62 (m, 1H), 2.97 (d, J = 17 Hz, 1H), 2.63 (dd, J = 17 Hz, 11 Hz, 1H), 1.59 (s, 9H), 1.56 (s, 9H).

APCI-MS $[M+H]^+$: 529.5

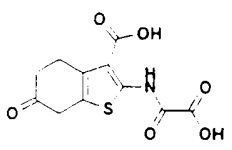
The above 2-(*tert*-butoxyoxalyl-amino)-5-(1-oxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (52 mg, 0.098 mmol) was treated with a solution of 50 % trifluoroacetic acid/dichloromethane (3 ml) for 4.5 h at room temperature. The volatiles were evaporated in vacuo and the residue chased three times with dichloromethane (10 ml). The solid formed was filtered off and washed with dichloromethane affording 28 mg (70 %) of the title compound as a solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 7.69 (d, J = 8 Hz, 1H), 7.61 - 7.59 (m, 2H), 7.51 - 7.45 (m, 1H), 4.81 (d, J = 15 Hz, 1H), 4.65 (d, J = 15 Hz, 1H), 4.60 (s, 2H), 3.95 - 3.92 (m, 1H), 3.75 (d, J = 5 Hz, 2H), 2.94 (d, J = 16 Hz, 1H), 2.56 (dd, J = 16 Hz, 10 Hz, 1H).

APCI-MS $[M+H]^+$: 417.3

HPLC (254.4nm): 3.079 s (100 %)

EXAMPLE 110



2-(Oxalyl-amino)-6-oxo-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid

2-(Ethoxyoxalyl-amino)-6-oxo-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid (3.0 g, 0.013 mol) was dissolved in a mixture of water (40 ml), ethanol (20 ml) and tetrahydrofuran (20 ml) at room temperature. To the resulting mixture was added 1 N sodium hydroxide (20.24 ml, 20.24 mmol). The resulting reaction mixture was stirred at room temperature for 72 h, pH was adjusted to 3 by addition of concentrated hydrochloric acid. The precipitate was filtered off and washed with water (2 x 15 ml), diethyl ether (2 x 15 ml) and dried in vacuo at 50 °C affording 1.96 g (73 %) of the title compound as a solid.

M.p.: > 230 °C

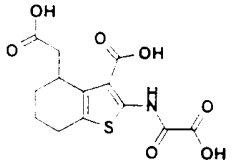
Calculated for $C_{11}H_9NO_6S$;

C, 46.64 %; H, 3.30 %; N, 4.94 %. Found:

C, 46.97 %; H, 3.30 %; N, 5.80 %.

By a similar procedure as described in Example 81 the following compounds have been prepared.

EXAMPLE 111



4-Carboxymethyl-2-(oxalyl-amino)-4,5,6,7-tetrahydro-benzo-[b]thienophene-3-carboxylic acid;

2-Carbmethoxymethylcyclohexanone was prepared in the same way as described in *J. Am. Chem. Soc.* **81**, 3955-3959 (1959) for 2-carbethoxy-methylcyclohexanone.

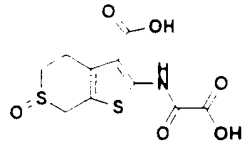
M.p.: > 250 °C

Calculated for $C_{13}H_{13}N_1O_7S_1$, 0.75 H_2O;

C, 45.81 %; H, 4.29 %; N, 4.11 %. Found:

C, 45.79 %; H, 4.02 %; N, 4,08 %.

EXAMPLE 112



2-(Oxalyl-amino)-6-oxo-4,7-dihydro-5H-thieno[2,3-c]thiopyran-3-carboxylic acid;

1-Oxo-2,3,5,6-tetrahydro-*4H*-thiopyran-4-one was prepared as described in *J. Org. Chem.* **27**, 282-284 (1962).

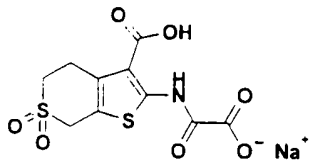
M.p.: > 250 °C.

Calculated for $C_{10}H_9N_1O_6S_2$, 0.2 x NaCl;

C, 38.13 %; H, 2.88 %; N, 4.45 %. Found:

C, 37.98 %; H, 2.82 %; N, 4,29 %.

EXAMPLE 113



2-(Oxalyl-amino)-6,6-dioxo-4,7-dihydro-5H-thieno[2,3-c]thiopyran-3-carboxylic acid, mono sodium salt;

1,1-Dioxide-2,3,5,6-tetrahydro-*4H*-thiopyran-4-one was prepared as described in *J. Org. Chem.* **60**, 1665-1673 (1995).

M.p.: > 250 °C

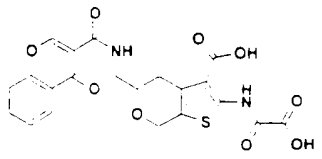
Calculated for $C_{10}H_8N_1O_7S_2Na_1$, 1 x H_2O;

C, 33.43 %, H, 2.81 %; N, 3.90 %. Found:

C, 33.43 %; H, 2.78 %; N, 3,76 %.

By a similar procedure as described in Example 107 the following compounds have been prepared.

EXAMPLE 114



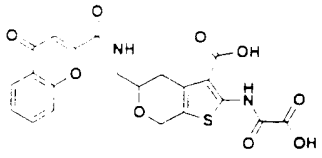
2-(Oxalyl-amino)-5-(((4-oxo-chromene-4H-3-carbonyl)amino)methyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 9.47 (t, J = 4 Hz, 1H), 9.08 (s, 1H), 8.19 (dd, J = 8 Hz, 2 Hz, 1H), 7.90 (dt, J = 8 Hz, 2 Hz, 1H), 7.78 (d, J = 8 Hz, 1H), 7.60 (t, J = 8 Hz, 1H), 4.88 (d, J = 15 Hz, 1H), 4.70 (d, J = 15 Hz, 1H), 3.83 - 3.79 (m, 1H), 3.72 - 3.66 (m, 1H), 3.55 - 3.48 (m, 1H), 2.95 (d, J = 15 Hz, 1H), 2.60 (dd, J = 15 Hz, 8 Hz, 1H).

LC/MS [M-H]$^-$: 471.4

HPLC (254.4 nm): 3.105 s, 94%.

EXAMPLE 115



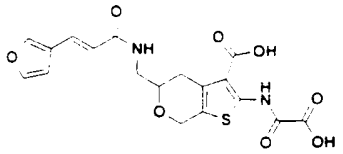
2-(Oxalyl-amino)-5-(((4-oxo-chromene-4H-2-carbonyl)amino)methyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 9.33 (t, J = 4 Hz, 1H), 8.05 (d, J = 8 Hz, 1H), 7.89 (t, J = 8 Hz, 1H), 7.76 (d, J = 8 Hz, 1H), 7.53 (t, J = 8 Hz, 1H), 6.84 (s, 1H), 4.83 (d, J = 15 Hz, 1H), 4.66 (d, J = 15 Hz, 1H), 3.89 - 3.84 (m, 1H), 3.56 - 3.45 (m, 2H), 2.98 (d, J = 18 Hz, 1H), 2.63 - 2.52 (m, 1H, partially obscured by DMSO).

LC/MS [M-H]$^-$: 471.4

HPLC (254.4 nm): 2.886 s, 95 %.

EXAMPLE 116



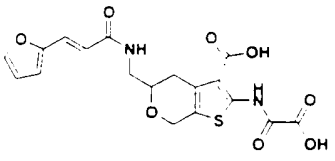
5-((3-Furan-3-yl-acryloylamino)-methyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 8.20 (t, J = 5 Hz, 1H), 7.99 (s, 1H), 7.71 (s, 1H), 7.33 (d, J = 15 Hz, 1H), 6.68 (s, 1H), 6.42 (d, J = 15 Hz, 1H), 4.81 (d, J = 15 Hz, 1H), 4.65 (d, J = 15 Hz, 1H), 3.74 - 3.67 (m, 1H), 3.44 - 3.34 (m, 2H), 2.91 (d, J = 17 Hz, 1H), 2.53 (dd, 1H, partially obscured by DMSO).

LC/MS [M-H]$^-$: 419.4

HPLC (254.4 nm): 2.822 s, 91%

EXAMPLE 117



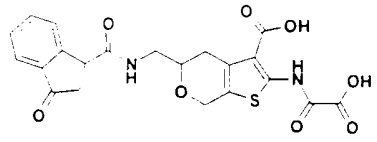
5-((3-Furan-2-yl-acryloylamino)-methyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 8.37 (t, 1H), 7.77 (s, 1H), 7.23 (d, J = 15 Hz, 1H), 6.76 (d, J = 3 Hz, 1H), 6.57 (dd, J = 3 Hz, 2 Hz, 1H), 6.50 (d, J = 15 Hz, 1H), 4.81 (d, J = 15 Hz, 1H), 4.65 (d, J = 15 Hz, 1H), 3.74 - 3.67 (m, 1H), 3.48 - 3.32 (m, 2H), 2.91 (d, J = 17 Hz, 1H), 2.53 (dd, 1H, partially obscured by DMSO).

$[M-H]^-$: 419.3

HPLC (254.4 nm): 2.815 s, 86%

EXAMPLE 118



2-(Oxalyl-amino)-5-(((3-oxo-indane-1-carbonyl)amino)methyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

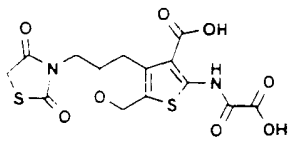
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 8.81 (bs, 1H), 7.74 - 7.62 (m, 3H), 7.47 (t, J = 7 Hz, 1H), 4.83 (d, J = 15 Hz, 1H), 4.67 (d, J = 15 Hz, 1H), 4.29 (t, J = 5 Hz, 1H), 3.41 - 3.25 (m, 3H), 2.91 (d, J = 15 Hz, 1H), 2.77 (d, J = 5 Hz, 2H), 2.58 - 2.51 (m, 1H, partially obscured by DMSO).

LC/MS $[M-H]^-$: 457.5

HPLC (254.4 nm): 2.634 s, 97 %.

By a similar procedure as described in Example 106 the following compound was prepared.

EXAMPLE 119



5-(2,4-Dioxo-thiazolidin-3-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

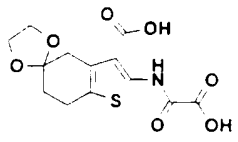
^{1}H NMR (400 MHz, CD_3OD and DMSO-d_6) δ 4.88 (m, 2H), 3.97 - 3.89 (m, 3H), 3.72 - 3.69 (m, 2H), 3.08 (m, 1H), 3.02 (m, 1H).

MS (ESI (-)): 399.

HPLC (254.4nm): 2.67, s, 100%.

By a similar procedure as described in Example 81 the following compounds have been prepared.

EXAMPLE 120



2-(Oxalyl-amino)-5-(2'-spiro[1',3']dioxolane)-6,7-dihydro-4H-benzo[b]thiophen-3-carboxylic acid;

M.p.: 232 - 234 °C

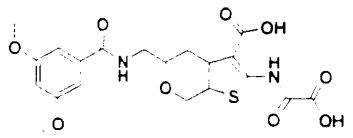
Calculated for $C_{13}H_{13}NO_7S$, 1 x H_2O;

C, 45.22 %; H, 4.38 %; N, 4.06 %. Found:

C, 45.24 %; H, 4.39 %; N, 3.98 %.

By a similar procedure as described in Example 107 the following compounds have been prepared

EXAMPLE 121



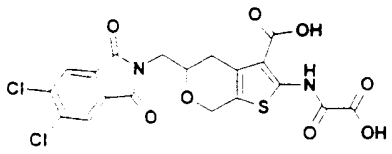
5-((3,5-Dimethoxy-benzoylamino)-methyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 8.63 (t, J = 5 Hz, 1H), 7.02 (s, 2H), 6.62 (s, 1H), 4.80 (d, J = 15 Hz, 1H), 4.64 (d, J = 15 Hz, 1H), 3.82 - 3.79 (m, 1H), 3.77 (s, 6H), 3.47 - 3.45 (m, 2H), 2.94 (d, J = 17 Hz, 1H), 2.53 (dd, J = 17 Hz, 11 Hz, 1H).

LC/MS [M-H]$^-$: 463.4

HPLC (254.4 nm): 3.161 s, 93%

EXAMPLE 122



5-(5,6-Dichloro-1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

To a solution of 2-hydroxymethyl-tetrahydro-pyran-4-one (625 mg, 4.81 mmol) in a mixture of pyridine (778 µl, 9.62 mmol) and chloroform (6.0 ml) at 0 °C under nitrogen was slowly added 4-nitrobenzenesulfonyl chloride (1.60 g, 7.22 mmol). The mixture was allowed to

warm to room temperature and stirred for 3 h. Chloroform (30 ml) was added and the solution washed with 2.0 N hydrochloric acid (3 x 10 ml), 5 % $NaHCO_3$ (3 x 10 ml) and water (3 x 10 ml). The organic phase was dried (Na_2SO_4), filtered and the solvent evaporated in vacuo. The solid residue was purified by column chromatography on silica gel using a gradient of dichloromethane:hexane:ethyl acetate (1:1:0 to 8:0:2) as eluent. Pure fractions were collected and the volatiles were evaporated in vacuo affording 0.98 g (65 %) of 4-nitro-benzenesulfonic acid 4-oxo-tetrahydro-pyran-2-ylmethyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (d, 2H, J = 7.8 Hz), 2.57 (m, 1H), 3.63 (m, 1H), 3.89 (m, 1H), 4.20 - 4.26 (m, 3H), 8.14 (dd, 2H, J = 0.6 Hz, J = 9 Hz), 8.42 (dd, 2H, J = 0.6 Hz, J = 9 Hz).

MS *m/z*: 315.3 (M+).

4-Nitro-benzenesulfonic acid 4-oxo-tetrahydro-pyran-2-ylmethyl ester (0.5 g, 1.59 mmol), ethylene glycol (986 mg, 15.9 mmol) and p-toluene sulfonic acid (61 mg, 0.32 mmol) were refluxed in benzene (20 ml) for 20 h. The solvent was removed in vacuo to afford a solid. The solid was dissolved in dichloromethane (30 ml) and successively washed with a saturated aqueous solution of sodium bicarbonate (2 x 5 ml) and water (2 x 5 ml). The organic phase was dried (Na_2SO_4), filtered and the solvent removal in vacuo afforded 582 mg (100 %) of 4-nitro-benzenesulfonic acid 1,4,8-trioxa-spiro[4.5]dec-7-ylmethyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 - 1.73 (m, 4H), 3.54 (m, 1H), 3.8 (m, 2H), 3.96 (m, 4H), 4.15 (m, 2H), 8.12 (dd, 2H, J = 1.5 Hz, J = 9.0 Hz), 8.40 (dd, 4H, J = 1.5 Hz, J = 9.0 Hz).

MS *m/z*: 359.3.

3.4-Dichlorophthalimide (90.2 mg, 0.42 mmol) was dissolved in N,N-dimethylformamide (2.0 ml) at room temperature. Sodium hydride (17 mg, 0.42 mmol) was added under nitrogen. 4-Nitro-benzenesulfonic acid 1,4,8-trioxa-spiro[4.5]dec-7-ylmethyl ester (100 mg, 0.28 mmol) was added and the mixture heated to 140 °C for 3 h. After cooling to room temperature the reaction mixture was added to ice water (5 ml) and the mixture extracted with ethyl acetate (3 x 15 ml). The combined ethyl acetate extracts were washed with 1.0 N hydrochloric acid (2 x 5 ml), water (2 x 5 ml), saturated sodium bicarbonate (2 x 5 ml) and water (2 x 5 ml). After drying (Na_2SO_4) followed by filtration, the solvent was removed in vacuo affording 97

mg (94 %) of 5,6-dichloro-2-(1,4,8-trioxa-spiro[4.5]dec-7-ylmethyl)-isoindole-1,3-dione as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (m, 2H), 1.78 (m, 2H), 3.54 (m, 1H), 3.64 (m, 1H), 3.88 (m, 2H), 3.95 (m, 4H), 7.95 (d, 2H, J = 3 Hz).

MS *m/z*: 373.7 (M+).

5,6-Dichloro-2-(1,4,8-trioxa-spiro[4.5]dec-7-ylmethyl)-isoindole-1,3-dione (87 mg, 0.234 mmol) was dissolved in tetrahydrofuran (2.5 ml). 1.0 N hydrochloric acid (1.0 ml) was added to the solution and the mixture was heated at 75 °C for 20 h. The heterogeneous mixture was evaporated to dryness in vacuo and the resulting solid was dissolved in dichloromethane (10 ml) and washed with water (3 x 2 ml). The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated in vacuo affording 62.1 mg (81 %) of 5,6-dichloro-2-(4-oxo-tetrahydro-pyran-2-ylmethyl)-isoindole-1,3-dione as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.31 - 2.41 (m, 2H), 2.48 (t, 1H, J = 2.0 Hz), 2.62 (m, 1H), 3.60 (m, 1H), 3.72 (m, 1H), 3.99 (m, 2H), 4.29 (m, 1H), 7.96 (d, 2H, J = 2.7 Hz).

MS *m/z*: 331.1 (M+).

5,6-Dichloro-2-(4-oxo-tetrahydro-pyran-2-ylmethyl)-isoindole-1,3-dione (60 mg, 0.18 mmol) was stirred with *tert*-butyl cyanoacetate (33.5 mg, 0.24 mmol), elemental sulfur (6.44 mg, 0.20 mmol) and morpholine (32.4 µl, 0.37 mmol) in ethanol for 20 h at 50 °C. The volatiles were evaporated in vacuo and the resulting solid was dissolved in dichloromethane (30 ml) and washed with water (2 x 10 ml). The organic phase was dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residue (111 mg) was purified by preparative TLC (Kieselgel 60F_{254}, 1 mm) using a mixture of hexane and ethyl acetate (1:1) as eluent. Pure compound was obtained after evaporation of the solvent in vacuo affording 28 mg (32 %) of 2-amino-5-(5,6-dichloro-1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 9H), 2.90 (m, 1H), 3.35 (m, 2H), 2.60 (m, 2H), 2.90 (m, 1H), 4.62 (m, 1H), 7.95 (d, 2H, J = 1.8 Hz).

MS m/z: 483.3 (M+), 427 (M-57).

A mixture of 2-amino-5-(5,6-dichloro-1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (27.5 mg, 0.057 mmol), imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (55.8 mg, 0.29 mmol) and triethylamine (16 µl, 0.114 mmol) in tetrahydrofuran (2 ml) was stirred at room temperature for 20 h. The volatiles were evaporated in vacuo and the resulting syrup was dissolved in dichloromethane (15 ml) and washed with water (3 x 3 ml). The organic phase was dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. The residue (35.7 mg) was purified by preparative TLC (Kieselgel $60F_{254}$, 0.5 mm) using a mixture of hexane and ethyl acetate (8:2) as eluent. After isolation 8.5 mg (24 %) of 2-(*tert*-butoxyoxalyl-amino)-5-(5,6-dichloro-1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester was obtained.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 (s, 18H), 2.68 (m, 1H), 2.97 - 3.02 (m, 1H), 3.82 (m, 1H), 4.63 - 4.68 (m, 1H), 4.77 - 4.82 (m, 1H), 7.97 (d, 2H, J = 2.1 Hz).

MS *m/z* 611.4 (M+).

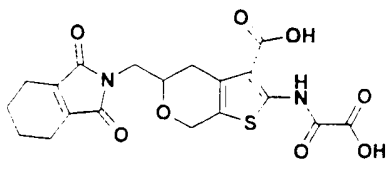
2-(*tert*-Butoxyoxalyl-amino)-5-(5,6-dichloro-1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (3.5 mg, 5.7x10-3 mmol) was dissolved in 20 % trifluoroacetic acid in dichloromethane (1.0 ml) and stirred for 2 h at room temperature. The volatiles were evaporated in vacuo which afforded 2.7 mg (95 %) of the title compound as a solid.

^{1}H NMR (400 MHz, CD_3OD) δ 2.66 (m, 1H), 3.10 (m, 1H), 3.80 (m, 1H), 3.98 (m, 2H), 4.66 (m, 1H), 4.74 (m, 1H).

MS *m/z* 498.3 (M-).

The following compounds were prepared in a similar way as described in example 122.

EXAMPLE 123



5-(1,3-Dioxo-1,3,4,5,6,7-hexahydro-isoindol-2-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid:

2-(1,4,8-Trioxa-spiro[4.5]dec-7-ylmethyl)-4,5,6,7-tetrahydro-isoindole-1,3-dione 73.1 mg (62 %) as an oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 - 1.58 (m, 2H), 2.24 (m, 2H), 2.62 (m, 2H), 3.10 (m, 2H), 3.50 (m, 2H), 3.71 (m, 3H), 3.94 (m, 6H), 5.9 (m, 2H).

2-(4-Oxo-tetrahydro-pyran-2-ylmethyl)-4,5,6,7-tetrahydro-isoindole-1,3-dione 50 mg (92 %) as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (m, 2H), 1.64 (m, 2H), 2.22 (m, 1H), 2.34 (m, 2H), 2.61 (m, 3H), 3.13 (m, 2H), 3.79 (m, 1H), 3.95 (m, 1H), 4.28 (m, 1H), 5.92 (m, 2H).

2-Amino-5-(1,3-dioxo-1,3,4,5,6,7-hexahydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester was obtained as a solid after purification by preparative TLC (Kieselgel $60F_{254}$, 1mm, hexane: ethyl acetate, 1:1) (36 mg, 47 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 9H), 2.22 (m, 2H), 2.62 (m, 2H), 2.83 (m, 1H), 3.11 (m, 2H), 3.56 (m, 1H), 3.83 (m, 2H), 4.50 (m, 2H), 5.89 (m, 2H).

MS *m/z* 419.5 (M+), 363.4 (M-57).

2-(*tert*-Butoxyoxalyl-amino)-5-(1,3-dioxo-1,3,4,5,6,7-hexahydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2.3-c]pyran-3-carboxylic acid *tert*-butyl ester was obtained after purification by preparative TLC (Kieselgel $60F_{254}$, 0.5 mm, hexane: ethyl acetate, 8:2).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 18H), 2.24 (m, 2H), 2.92 (m, 3H), 3.14 (m, 2H), 3.90 (m, 2H), 4.11 (m, 1H), 4.63 (m, 1H), 4.78 (m, 1H), 5.91 (m, 2H).

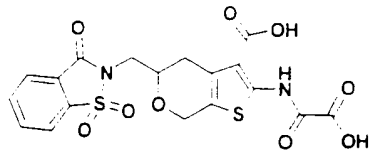
MS *m/z* 545.4 (M-), 489.4 (M-57).

The title compound was obtained as a solid (17.2 mg, quantitative yield).

^{1}H NMR (400 MHz, CD_3OD) δ 2.28 (m, 2H), 2.55 (m, 2H), 2.97 (m, 2H), 3.31 (m, 2H), 3.56 - 3.93 (m, 3H), 4.70 (m, 2H), 5.91 (m, 2H).

MS *m/z* 433.3 (M-).

EXAMPLE 124



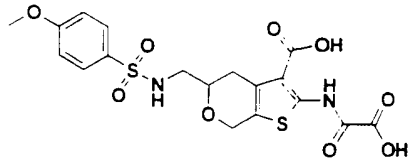
2-(Oxalyl-amino)-5-(1,1,3-trioxo-1,3-dihydro-1H-benzo[d]isothiazol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

^{1}H NMR (400 MHz, CD_3OD) δ 8.09 - 7.8 (m, 4H), 4.85 - 4.67 (m, 3H), 4.21 - 4.12 (m, 1H), 4.02 - 3.94 (m, 1H), 3.11 - 3.06 (m, 1H), 2.90 - 2.80 (m, 1H).

MS (ESI (-)): 465.

HPLC (254.4nm): 2.31, s, 99%.

EXAMPLE 125



5-[(4-Methoxy-benzenesulfonylamino)-methyl]-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

To a solution of 2-amino-5-aminomethyl-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (101 mg, 0.35 mmol) in dichloromethane (1 ml) was added pyridine (32 µl, 0.39 mmol) and 4-methoxybenzenesulfonyl chloride (82 mg, 0.39 mmol). The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was diluted with dichloromethane (2 ml) and subjected to preparative TLC (1:1 hexanes/ethyl acetate) affording

10 mg, (10 %) of 2-amino-5-((4-methoxy-benzenesulfonylamino)-methyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 9Hz, 2H), 6.93 (d, J = 9Hz, 2H), 5.3 (bs, 2H), 4.57 (s, 2H), 3.84 (s, 3H), 3.72 (m, 1H), 3.10 - 3.06 (m, 1H), 2.95 - 2.87 (m, 1H), 2.69 - 2.64 (m, 1H), 2.41 - 2.32 (m, 1H), 1.47 (s, 9H).

MS: APCI (-): 453 [M-H].

To a solution of 2-amino-5-((4-methoxy-benzenesulfonylamino)-methyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (8 mg, 0.017 mmol) in dichloromethane (1 ml) was added triethylamine (7.4 µl, 0.051 mmol), and imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (10 mg, 0.051 mmol) and stirred at room temperature for 16 h. The volatiles were removed in vacuo and to the residue was added dichloromethane (2 ml). The solution was purified by preparative TLC (10 % methanol/90 % dichloromethane) affording 10 mg (100 %) of 2-(*tert*-butoxyoxalyl-amino)-5-((4-methoxy-benzenesulfonylamino)-methyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 9 Hz, 2H), 6.93 (d, J = 9 Hz, 2H), 4.68 (m, 2H), 3.85 (s, 3H), 3.7 (m, 3H), 3.29 - 3.22 (m, 1H), 2.80 - 2.75 (m, 1H), 2.53 - 2.43 (m, 1H), 1.56 (s, 18H).

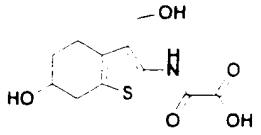
MS:APCI (+): 582.8 [M+H], 527 (-1 *tert*-Bu).

2-(*tert*-Butoxyoxalyl-amino)-5-((4-methoxy-benzenesulfonylamino)-methyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (10 mg, 0.017 mmol) was added to a solution of 25 % trifluoroacetic acid in dichloromethane (2 ml). The reaction mixture was stirred at room temperature for 2 h, at which time, the solvent was removed in vacuo. The residue was precipitated by addition of diethyl ether and washed two times with diethyl ether affording after drying 2 mg (25 %) of the title compound as a solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.78 (d, J = 9 Hz, 2H), 7.02 (d, J = 9 Hz, 2H), 4.76 - 4.63 (m, 2H), 3.84 (s, 3H), 3.75 (m, 1H), 3.50 - 3.47 (m, 2H), 2.89 - 2.83 (m, 1H), 2.52 - 2.42 (m, 1H).

MS: APCI (+): 471 [M+H];

EXAMPLE 126



N-(6-Hydroxy-3-hydroxymethyl-4,5,6,7-tetrahydro-benzo[b]thiophen-2-yl)-oxalamic acid:

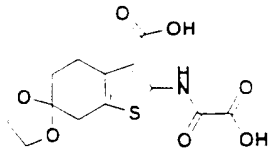
2-(Ethoxyoxalyl-amino)-6-(2'-spiro[1',3']dioxolane)-6,7-dihydro-4H-benzo[b]thiophen-3-carboxylic acid *tert* butyl ester (20 g, 0.05 mol) was dissolved in a (1:4) mixture of trifluoroacetic acid and dichloromethane (200 ml) containing water (1 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 20 h. The volatiles were evaporated in vacuo and the solid residue was trituated with diethyl ether (2 x 100 ml) and dried in vacuo affording 15.08 g (100 %) of 2-(ethoxyoxalyl-amino)-6-oxo-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carboxylic acid as a solid.

To a mixture of ethanol (50 ml) and dichloromethane (50 ml) was added 2-(ethoxyoxalyl-amino)-6-oxo-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carboxylic acid (2.0 g, 6.43 mmol) followed by sodium borhydride (124 mg, pellets). The resulting mixture was stirred at room temperature for 1 h and an additional sodium borhydride pellet was added. After stirring for an addition 4 h the reaction mixture was quenched by addition of a mixture of water (100 ml) and formic acid (100 ml) at 0 °C. The aqueous phase was extracted with ethyl acetate (2 x 100 ml) and the combined organic phases were washed with brine (100 ml), dried Na_2SO_4, filtered and evaporated in vacuo affording 860 mg (43 %) of the title compound as a solid. After standing for 18 h the aqueous phase was filtered and the filter cake was washed with water (2 x 15 ml), diethyl ether (2 x 15 ml) and dried in vacuo affording an additional portion 710 mg (48 %) of the title compound as a solid.

Calculated for $C_{11}H_{13}N_1O_5S_1$, 0.5 x H_2O
C, 47.14 %; H, 5.03 %; N, 5.00 %. Found:
C, 47.19 %; H, 5.00 %; N, 4.94 %.

The following compound was prepared in a similar way as described in example 81.

EXAMPLE 127



2-(Oxalyl-amino)-6-(2'-spiro[1',3']dioxolane)-6,7-dihydro-4H-benzo[b]thiophen-3-carboxylic acid;

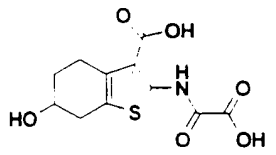
M.p.: > 250 °C.

Calculated for $C_{13}H_{13}NO_7S$;

C, 47.70 %. H, 4.00 %; N, 4.28 %. Found:

C, 47.93 %. H, 4.09 %; N, 4.27 %.

EXAMPLE 128



6-Hydroxy-2-(oxalyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-carboxylic acid;

2-(Ethoxyoxalyl-amino)-6-(2'-spiro[1',3']dioxolane)-6,7-dihydro-4H-benzo[b]thiophen-3-carboxylic acid ethyl ester (8.7 g, 22.7 mmol) was dissolved in a ice bath cooled mixture of 25 % trifluoroacetic acid in dichloromethane (100 ml) and water (0.5 ml) was added. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 48 h. The volatiles were evaporated in vacuo and the residue dissolved in ethanol (100 ml) and evaporated in vacuo (2 times). The solid residue was washed with diethyl ether (80 ml) and dried in vacuo at 50 °C affording 6.68 g (88 %) of 2-(ethoxyoxalyl-amino)-6-oxo-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester as a solid.

To a solution of 2-(ethoxyoxalyl-amino)-6-oxo-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester (2.0 g, 5.89 mmol) in a mixture of dichloromethane (40 ml) and ethanol (40 ml) was added sodium borohydride (64 mg, 1.77 mmol). The reaction mixture

was stirred at room temperature for 64 h, additional sodium borohydride (22.3 mg, 0.59 mmol) was added and stirring was continued for an additional 18 h. Two more portions of sodium borohydride (23 mg and 15 mg) was added during the next 6 h of stirring. To the reaction mixture was added ice cooled saturated ammonium chloride (50 ml) and the resulting mixture was extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was dissolved twice in ethyl acetate (100 ml) and evaporated in vacuo. The solid residue was washed with diethyl ether (80 ml) and dried in vacuo at 50 °C affording 1.46 g (75 %) of 2-(ethoxyoxalyl-amino)-6-hydroxy-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester as a solid. 1.35 g of this material was subjected to column chromatography (slilca gel) using a mixture of ethyl acetate and heptane (1:1) as eluent. Pure fractions were collected and the solvent evaporated in vacuo affording 0.9 g of pure 2-(ethoxyoxalyl-amino)-6-hydroxy-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester as a solid.

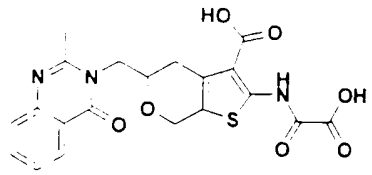
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (m, 6H), 1.86 (m, 2H), 2.02 (m, 1H), 2.71 (dd, 1H), 2.85 (m, 1H), 3.00 (m, 2H), 4.19 (bs, 1H), 4.40 (dq, 4H), 12.45 (bs, 1H, N*H*CO).

To a solution of the above di-ethyl ester (0.3 g, 0.88 mmol) in water (10 ml) was added 1 N sodium hydroxide (3.1 ml, 3.08 mmol). The resulting reaction mixture was stirred at room temperature for 16 h. The aqueous phase was acidified by addition of concentrated hydrochloric acid to pH = 1 and the reaction mixture was evaporated in vacuo to ½ the original volume. The precipitate was filtered off, washed with a small portion of diethyl ether and dried in vacuo at 50 °C for 16 h affording 130 mg (52 %) of the title compound as a solid. M.p.: amorph

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.63 (m, 1H), 1.86 (m, 1H), 2.5 (m, 1H, partly obscured by DMSO), 2.71 (m, 1H), 2.86 (m, 2H), 3.91 (m, 1H), 4.87 (bs, 1H), 12.35 (bs, 1H, N*H*CO).

The following compound was prepared in a similar way as described in example 107.

EXAMPLE 129



5-(2-Methyl-4-oxo-4H-quinazolin-3-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid:

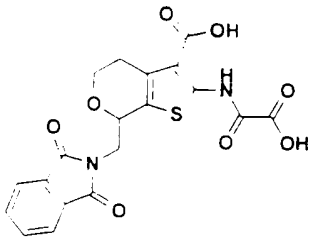
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 8.10 (d, J = 8 Hz, 1H), 7.80 (t, J = 7 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 7.49 (t, J = 7 Hz, 1H), 4.78 (d, J = 15 Hz, 1H), 4.53 (d, J = 15 Hz, 1H), 4.39 (d, J = 15 Hz, 1H), 4.21 (dd, J = 15 Hz, 9Hz, 1H), 4.00 - 3.94 (m, 1H), 3.05 (d, J = 17 Hz, 1H), 2.74 - 2.65 (m, 1H, partially obscured by neighboring singlet), 2.68 (s, 3H).

^{13}C NMR (100.6 MHz, DMSO-d_6) δ 167.7, 162.8, 161.6, 157.6, 156.1, 148.3, 146.9, 136.0, 130 5, 127.9, 127.8, 126.5, 121.4, 115.0, 74.4, 65.9, 49.8, 31.4, 25.0.

[M-H]$^-$: 442.1

HPLC (254.4 nm): 2.631 s, 81 %.

EXAMPLE 130



7-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid:

Phthalimidoacetaldehyde diethyl acetal (100 g, 0.38 mol) and 1 N hydrochloric acid (600 ml) was mixture was stirred at reflux temperature for 5 min. or until a homogeneous solution is obtained. The reaction mixture was cooled and the precipitate was filtered off and dried in vacuo at 50 °C for 16 h which afforded 63.3 g (88 %) of phthalimido-acetaldehyde as a solid.
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.58 (s, 2H), 7.76 - 7.78(m, 2H), 7.90 - 7.92 (m, 2H), 9.67 (s, 1H).

To a mixture of phthalimidoacetaldehyde (64 g, 0.34 mol) and trans-1-methoxy-3-(trimethylsilyloxy)-1,3-butadiene (81.5 g, 0.38 mol) in benzene (600 ml) stirred for 15 min. under nitrogen was added dropwise a 45 % solution of zinc chloride diethyl ether complex in dichloromethane (55.5 ml, 0.17 mol) at 0 °C. The reaction was allowed warm up to room temperature overnight. To the reaction mixture was added water (500 ml) and the resulting mixture was extracted with ethyl acetate (200 ml). The organic extract was washed successively with 1.0 N hydrochloric acid (2 x 200 ml) and brine (200 ml). The organic phase was dried (Na_2SO_4), filtered and the solvent evaporated in vacuo which afforded a slowly crystallising oil (98 g). To the solid was added a mixture of ethyl acetate and diethyl ether (400 ml, 1:1) and the resulting precipitate was filtered off, washed with a small portion of diethyl ether and dried at 50 °C for 1h affording 59.8 g (69 %) of 2-(4-oxo-3,4-dihydro-2H-pyran-2-ylmethyl)-isoindole-1,3-dione as a solid. The filtrate was evaporated in vacuo and the residue purified by column chromatography on silica gel (1 L) using a mixture of ethyl acetate and heptane (1:2) as eluent. Pure fractions were collected and the solvent evaporated in vacuo to almost dryness, the solid was filtered off and dried in vacuo at 50 °C for 16 h affording an additional 15 g (17 %) of 2-(4-oxo-3,4-dihydro-2H-pyran-2-ylmethyl)-isoindole-1,3-dione as a solid.
^{1}H NMR (300 MHz, $CDCl_3$) δ 2.61 (d, 2H), 3.85 (dd, 1H), 4.18 (dd, 1H), 4.76 (m, 1H), 5.43 (d, 1H), 7.28 (d, 1H), 7.69 - 7.77 (m, 2H), 7.84 - 7.88 (m, 2H).

2-(4-Oxo-3,4-dihydro-2H-pyran-2-ylmethyl)-isoindole-1,3-dione (13 g, 0.051 mol) was dissolved in ethyl acetate (250 ml) and placed in a Parr bottle. 10 % Pd/C (1.5 g) was carefully added and the mixture was shaken under a pressure of 30 psi of hydrogen for 6.5 h (Parr apparatus). Filtration followed by evaporation of the ethyl acetate in vacuo afforded a crude 11.5 g of 2-(4-oxo-tetrahydro-pyran-2-ylmethyl)-isoindole-1,3-dione pure enough for the next

step. Analytical pure compound could be obtained by purification of a small sample (250 mg) by column chromatography on silica gel, utilising hexane/ethyl acetate as a gradient (from 100/0 to 50/50). Pure fractions were collected and the solvent evaporated in vacuo affording 142 mg (55 %) 2-(4-oxo-tetrahydro-pyran-2-ylmethyl)-isoindole-1,3-dione as a solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.30 - 2.68 (m, 4H), 3.62 (m, 1H), 3.74 (m, 1H), 4.00 (m, 2H), 7.75 (m, 2H), 7.88 (m, 2H).

To a mixture of 2-(4-oxo-tetrahydro-pyran-2-ylmethyl)-isoindole-1,3-dione (18.7 g, 0.072 mol), *tert*-butyl cyanoacetate (11.2 g, 0.079 mol) and elemental sulfur (2.5 g, 0.079 mol) in ethanol was added morpholin (20 ml) and the resulting mixture was stirred at 50 °C for 3 h. The cooled reaction mixture was filtered and the volatiles were evaporated in vacuo. To the residue was added water (200 ml) and diethyl ether 100 ml. A precipitate was filtered off and dried in vacuo at 50 °C affording 9.1 g (30 %) of 2-amino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.
The filtrate was extracted with ethyl acetate (2 x 150 ml) and washed with brine (100 ml), dried (Na_2SO_4), filtered and the solvent evaporated in vacuo. The residue (20 g) was purified by column chromatography on silica gel (1 L) using as mixture of hexane and ethyl acetate (1:2) as eluent. Pure fractions were collected and the solvent evaporated in vacuo. The residue was washed with diethyl ether and the solid was filtered off and dried in vacuo at 50 °C affording an additional 2.2 g (7 %) of 2-amino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.
The filtrate was evaporated in vacuo affording almost pure 10.2 g (34 %) of 2-amino-7-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as an oil.

2-amino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 2.54 - 2.63 (m, 1H), 2.84 - 2.90 (m, 1H), 3.79 (q, 1H), 3.96 - 4.04 (m, 2H), 4.48 - 4.62 (m, 2H), 5.91 (bs, 2H, NH_2), 7.70 (m, 2H), 7.84 (m, 2H).

2-amino-7-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 2.71 - 2.90 (m, 2H), 3.67 - 3.77 (m, 2H), 4.02 - 4.15 (m, 2H), 4.90 (m, 1H), 6.04 (bs, 2H, NH_2), 7.70 (m, 2H), 7.84 (m, 2H).

A mixture of 2-amino-7-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (10.2 g, 0.25 mol), imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (7.2 g, 0.037 mol) in dry tetrahydrofuran (150 ml) was stirred at room temperature for 4 h. An additional portion of imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (2.0 g, 0.01 mol) was added and the resulting mixture was stirred for 16 h at room temperature. The precipitate was filtered off and washed with small portions of diethyl ether and dried in vacuo affording 3.5 g (26 %) of 2-(*tert*-butoxyoxalyl-amino)-7-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

The filtrate was evaporated in vacuo and to the residue was added water (100 ml) and ethyl acetate (100 ml). The precipitate was filtered off and dried in vacuo at 50 °C affording an additional 0.8 g (6 %) of 2-(*tert*-butoxyoxalyl-amino)-7-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60 (s, 9H), 1.62 (s, 9H), 2.79 - 2.97 (m, 2H), 3.73 (m, 1H), 3.83 - 3.88 (dd, 1H), 4.07 - 4.16 (m, 2H), 5.09 (m, 1H), 7.71 (m, 2H), 7.85 (m, 2H), 12.55 (bs, 1H, N*H*CO).

The above 2-(*tert*-butoxyoxalyl-amino)-7-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (0.8 g, 1.47 mmol) was added to a solution of 25 % trifluoroacetic acid in dichloromethane (30 ml). The reaction mixture was stirred at room temperature for 6 h, at which time, the solvent was removed in vacuo. The residue was precipitated by addition of diethyl ether, filtered off and dried in vacuo at 50 °C affording 0.5 g (79 %) of the title compound as a solid.

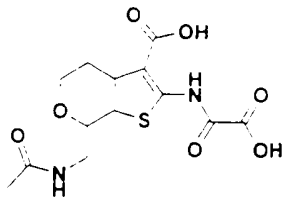
M.p.: > 250 °C.

Calculated for $C_{19}H_{14}N_2O_8S$, 0.5 x H_2O;

C, 51.94 %; H, 3.44 %; N, 6.38 %. Found:

C, 52.02 %; H, 3.37 %; N, 6.48 %.

EXAMPLE 131



7-(Acetylamino-methyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid;

To a mixture of 2-amino-7-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (6.0 g, 0.014 mol) in ethanol (100 ml) was added hydrazine hydrate (1.4 ml, 0.028 mol). The reaction mixture was heated at reflux for 1 h, cooled and the precipitate filtered off. The filtrate was evaporated in vacuo and to the residue was added water (100 ml) and the resulting mixture was extracted with diethyl ether (2 x 100 ml). The combined organic extracts were washed with brine (100 ml), dried (Na_2SO_4), filtered and the solvent evaporated in vacuo affording 2.9 g (71 %) of 2-amino-7-aminomethyl-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as an oil.
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (s, 9H), 2.70 - 2.97 (m, 4H), 3.69 - 3.78 (m, 1H), 4.13 (m, 1H), 4.50 (m, 1H), 6.09 (bs, 2H, thiophen-N*H*$_2$).

To a ice water cooled solution of the above 2-amino-7-aminomethyl-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (1.5 g, 5.27 mmol) and triethylamine (1.5 ml) in dichloromethane (50 ml) was added dropwise acetylchloride (0.46 g, 5.80 mmol). The reaction mixture was allowed to reach room temperature and stirred for an additional 0.5 h. The reaction mixture was washed with water (2 x 25 ml), dried (Na_2SO_4), filtered and the solvent evaporated in vacuo. The residue was purified by column chromatography on silicagel (1 L) using first ethyl acetate and later on a mixture of ethyl acetate and ethanol (20:1) as eluents. Pure fractions were collected and the solvent evaporated in vacuo affording 0.3 g (17 %) of 7-(acetylamino-methyl)-2-amino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56 (s, 9H), 1.99 (s, 3H), 2.77 (m, 2H), 3.19 (m, 1H), 3.67 - 3.79 (m, 2H), 4.09 - 4.16 (m, 1H), 4.63 (m, 1H), 5.91 (bs, 1H), 6.10 (bs, 2H).

To a mixture of the above 7-(acetylamino-methyl)-2-amino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (0.3 g, 0.92 mmol) in dry tetrahydrofuran (40 ml) was added dropwise a mixture of imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (0.22 g, 1.10 mmol) in dry tetrahydrofuran (5 ml). The mixture was stirred at room temperature for 3 h. The volatiles were evaporated in vacuo and the residue was dissolved in ethyl acetate (100 ml) and washed with water (50 ml) and brine (50 ml). The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. The residue (0.4 g) was stirred with a mixture of diisopropyl ether (5 ml) and diethyl ether (5 ml). The precipitate was filtered off and the filtrate evaporated in vacuo affording 0.25 g (60 %) of 7-(acetylamino-methyl)-2-(*tert*-butoxyoxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as an oil.

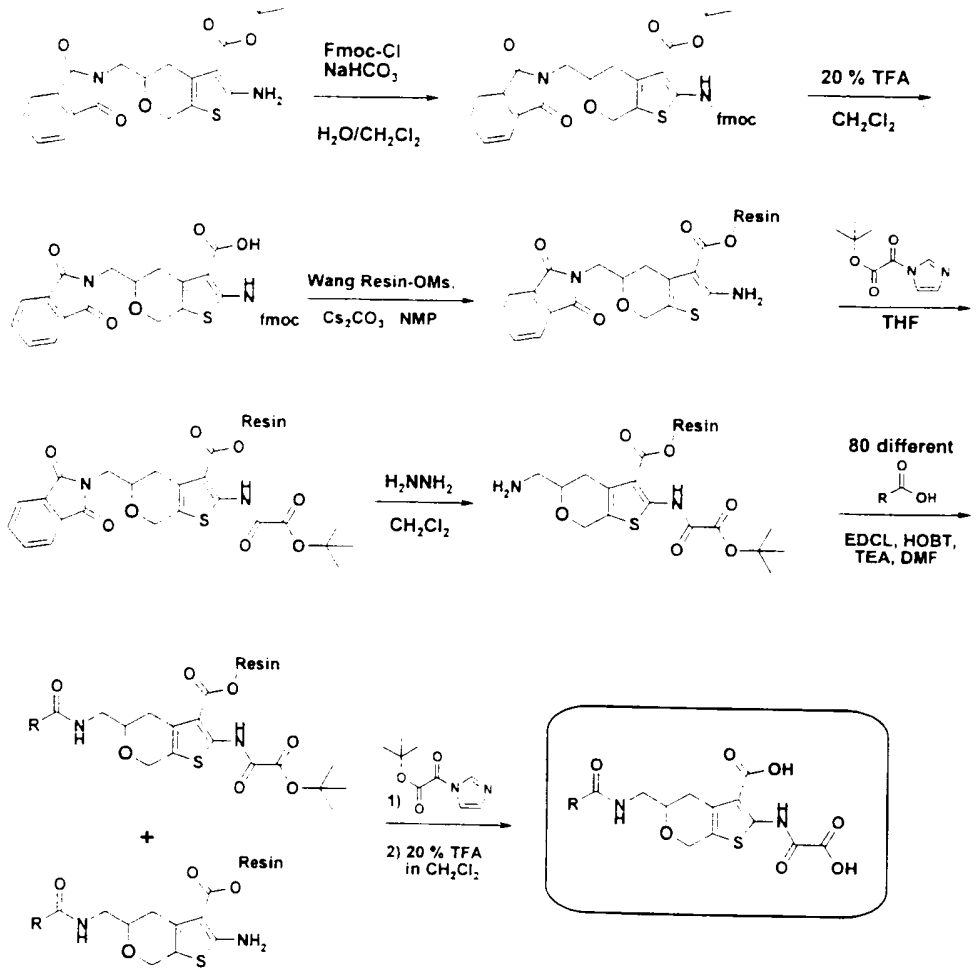
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.64 (s, 9H), 1.65 (s, 9H), 2.02 (s, 3H), 2.87 (m, 2H), 3.29 (m, 1H), 3.74 (m, 1H), 3.89 (ddd, 1H), 4.18 (m, 1H), 4.78 (m, 1H), 5.93 (bs, 1H, N*H*COMe), 12.5 (s, 1H, N*H*COCOOH).

The above 7-(acetylamino-methyl)-2-(*tert*-butoxyoxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (0.2 g, 0.44 mmol) was added to a solution of 25 % trifluoroacetic acid in dichloromethane (20 ml). The reaction mixture was stirred at room temperature for 4 h. at which time, the solvent was removed in vacuo. The residue was precipitated by addition of diethyl ether, filtered off and dried in vacuo at 50 °C affording 0.11 g (73 %) of the title compound as a solid.

M.p.: 220 - 222 °C.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.87 (s, 3H), 2.82 (bs, 2H), 3.19 (m, 1H), 3.51 (m, 1H), 3.67 (m, 1H), 4.07 (m, 1H), 4.69 (m, 1H), 8.14 (t, 1H, N*H*COMe), 12.3 (s, 1H, N*H*COCOOH).

EXAMPLE 132



To 2-amino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester (4.5 g, 0.011 mole) dissolved in dichloromethane (30 ml), was added sodium bicarbonate (1.0 g, 0.011 mole) dissolved in water (16 ml). The reaction mixture was cooled to 0 °C and 9-fluorenylmethyl chloroformate (3.0 g, 0.012 mole) was added. After stirring for 5 minutes the reaction mixture was warmed to room temperature and stirred vigorously for 16 h. The organic layer was separated and washed with brine (10 ml). The aqueous phase was extracted with dichloromethane (2 x 20 ml) and the combined organic phases were dried ($MgSO_4$), filtered and evaporated in vacuo to give an orange solid which was purified by flash chromatography using dichloromethane as eluent. Pure fractions

were collected and evaporated in vacuo affording 5.6 g (81 %) of 5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid *tert*-butyl ester as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.60 (bs, 1H), 7.87 - 7.84 (m, 2H), 7.75 (d, J = 8 Hz, 2H), 7.73 - 7.70 (m, 2H), 7.60 (d, J = 8 Hz, 2H), 7.39 (t, J = 8 Hz, 2H), 7.30 (t, J = 8 Hz, 2H), 4.74 (d, J = 14 Hz, 1H), 4.62 (d, J = 14 Hz, 1H), 4.48 (d, J = 7 Hz, 2H), 4.27 (t, J = 7 Hz, 1H), 4.05 - 4.00 (m, 2H), 3.86 - 3.80 (m, 1H), 2.92 (d, J = 17 Hz, 1H), 2.64 (dd, J = 17, 9 Hz, 1H), 1.52 (s, 9H).

LC/MS $[M+H]^+$: 637.49

The above F-moc protected thieno[2,3-c]pyran (5.5 g, 8.6 mmole) was added at 0 °C to a solution of 20 % trifluoroacetic acid in dichloromethane (30 ml). The reaction was stirred for 4 h at room temperature. The volatiles were evaporated in vacuo and the residue was precipitated with diethyl ether, filtered off and dried, which afforded 4.2 g (85 %) of 5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(9H-fluoren-9-ylmethoxy-carbonylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid as a solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (br s, 1H), 7.88 (d , J = 5 Hz, 2H), 7.88 - 7.82 (m, 4H), 7.66 (d, J = 5 Hz, 2H), 7.40 (t, J = 5 Hz, 2H), 7.32 (t, J = 5 Hz, 2H), 4.68 - 4.48 (m, 4H), 4.34 (t, J = 5 Hz, 1H), 3.90 - 3.81 (m, 2H), 3.72 - 3.67 (m, 1H), 2.87 (m, 1H), 2.51 (m, 1H).

To Wang-Resin (3.75 g, 4.5 mmol) was added dichloromethane (50 ml) and the mixture was cooled to 0 °C under nitrogen. Diisopropylethylamine (25 ml) was added followed by methanesulfonyl chloride (2.25 ml, 29 mmol). The reaction was stirred at 0 °C for 0.5 h, then at room temperature for another 0.5 h. The resin was filtered off and washed with dichloromethane (2 x 30 ml), N-methylpyrrolidinone (20 ml) and again with dichloromethane (2 x 30 ml). The Wang-resin methansulfonyl ester was dried in vacuo for 2 h and used directly in the next step.

To the above Wang-Resin methansulfonyl ester and 5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(9H-fluoren-9-ylmethoxy-carbonylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid (4.85 g, 8.4 mmol) was added N-methylpyrrolidinone (45 ml). Cesium car-

bonate (2.2 g, 6.7 mmol) was added and the reaction stirred under nitrogen for 16 h and then at 80 °C for 36 h. The mixture was cooled to room temperature, the resin filtered off, washed with water, methanol, and dichloromethane repeatedly and dried in vacuo for 2 h affording 5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-2-(9H-fluoren-9-ylmethoxy-carbonylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid Wang-Resin ester.

The above Wang-Resin ester (4.85 g) was stirred in a solution of 20 % piperidine in tetrahydrofuran (20 ml) for 45 minutes. The resin was then filtered off, washed with tetrahydrofuran (2 x 20 ml), methanol (2 x 20 ml), and dichloromethane (3 x 20 ml) and dried in vacuo for 3 h affording 2-amino-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid Wang-Resin ester.

The above Wang-Resin ester (4.85 g) was suspended in a mixture of dichloromethane (50 ml) and triethylamine (3.0 ml). Imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (4.2 g, 0.021 mol) was added under nitrogen and the reaction stirred at room temperature for 16 h. The resin was filtered off, washed with methanol (30 ml) then dichloromethane (30 ml) and this process was repeated twice. The resin was dried in vacuo for several hours affording 2-(*tert*-butoxyoxalyl-amino)-5-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid Wang Resin.

A small sample of the above Wang-Resin ester was treated with 20 % trifluoroacetic acid in dichloromethane (3 ml) for 1 h. The resin was filtered off and the filtrate concentrated in vacuo. The residue was evaporated twice from dichloromethane yielding 30 mg of a solid, which had ^{1}H NMR and MS consistent with the compound synthesized in example 106. The loading of the Wang-Resin was thus determined to be 0.6 mmol/g.

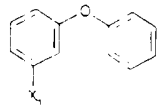
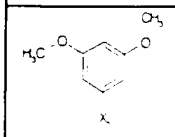
The above Wang Resin ester (3.0 g, 1.8 mmol) was suspended in dichloromethane (25 ml). Hydrazine (0.14 ml, 4.5 mmol) was added and the reaction stirred under nitrogen at room temperature for 24 hours. The resin was filtered off and washed multiple times, alternating between methanol and dichloromethane. The filtrate was collected and concentrated to yield 260 mg of a solid. The reaction was determined to be incomplete by analysis of the byproduct, at which time the resin was suspended again in dichloromethane (15 ml) and treated with hydrazine (50 µl) for an additional 16 h. The resin was filtered off and washed as before, yielding an additional 30 mg of byproduct from the filtrate. At this point the reaction was

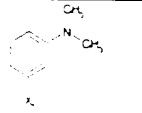
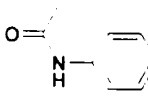
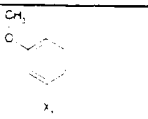
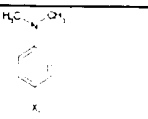
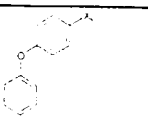
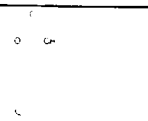
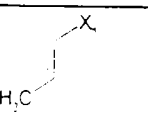
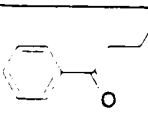
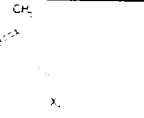
judged to be complete and the resin was dried in vacuo for 3 h, yielding 2.67 g of 5-aminomethyl-2-(*tert*-butoxyoxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid Wang-Resin. The resin gave a positive ninhydrin test for amines.

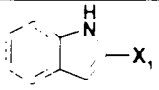
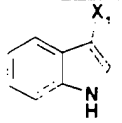
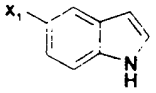
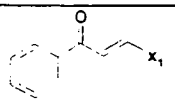
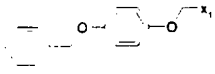
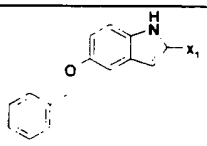
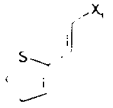
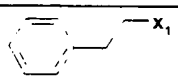
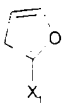
The above Wang Resin ester (2.67 g) was suspended in a mixture of tetrahydrofuran and dichloromethane (1:1, 90 ml) and distributed to the OntoBlock (80 wells, 0.02 mmol per well). The blocks were drained. Meanwhile, 80 carboxylic acids were weighed into individual vials (0.044 mmol per vial). A solution of 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.85 g, 4.4 mmol), 1-hydroxy-benzotriazole hydrate (0.6 g, 4.4 mmol), and triethylamine (1.1 ml, 8.0 mmol) was prepared in N,N-dimethylformamide (100 ml). This solution was added to each vial (1 ml per vial) and then the contents of each vial were transferred to a well of the OntoBlock (occasionally the vials were sonicated to achieve full solubility). The blocks were then shooked for 2 days. After this time the blocks were drained and washed using methanol and dichloromethane. The blocks were then placed in a vacuum dessicator for 2 h, after which 1 ml of a solution of imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (0.2 M in dichloromethane) was added to each well. The blocks were then shooked for 16 h. Again the blocks were washed using the above method. After washing, 1 ml of a solution of 20 % trifluoroacetic acid in dichloromethane was added to each well and allowed to sit for 45 minutes. The block was drained and the filtrates collected in a microtiter plate. The wells were treated with an additional 0.5 ml solution of 20 % trifluoroacetic acid in dichloromethane and the filtrate again collected. The volatiles were evaporated in vacuo, yielding 80 compounds as solids in the microtiter plate. The plate was analyzed by Mass Spectrometry in which 66 of the wells showed the expected product as the molecular ion.

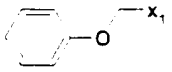
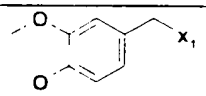
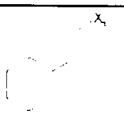
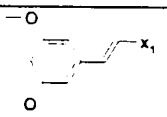
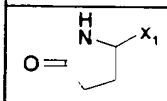
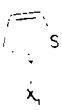
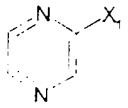
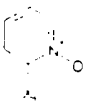
X_1 is point of attachment.

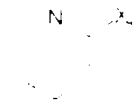
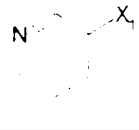
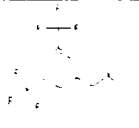
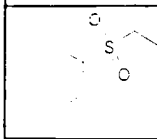
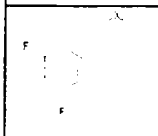
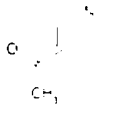
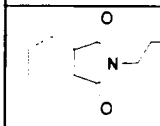
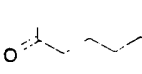
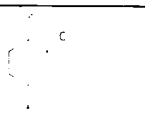
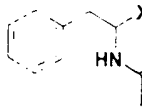
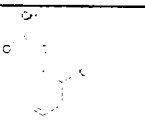
The percentage means the area of the peak of the HPLC at 220 nm.

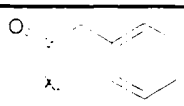
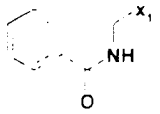
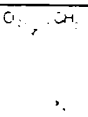
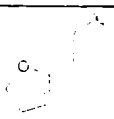
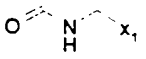
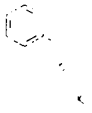
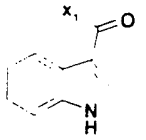
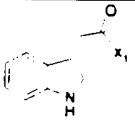
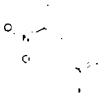
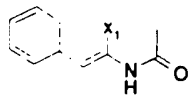
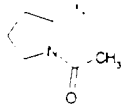
R	Formula	Mw	LC/MS
	$C_{24}H_{20}N_2O_8S$	496,50	495 (M-H,21%)
	$C_{20}H_{20}N_2O_9S$	464,45	463 (M-H,30%)

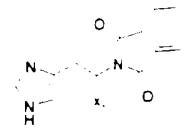
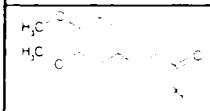
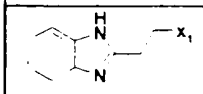
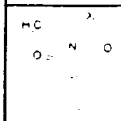
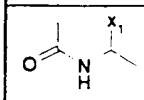
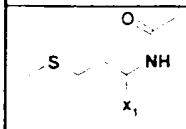
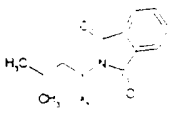
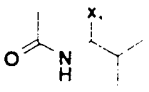
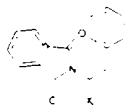
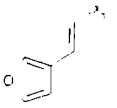
	C20H14F6N2O7S	540.40	539 (M-H,16%)
	C18H22N2O7S	410.45	409 (M-H,33%)
	C20H21N3O7S	447.47	446 (M-H,39%)
	C20H19N3O8S	461,45	460 (M-H,38%)
	C18H20N2O7S	408,43	407 (M-H,40%)
	C19H18N2O8S	434,43	433 (M-H,49%)
	C20H21N3O7S	447,47	446 (M-H,38%)
	C24H20N2O8S	496,50	495 (M-H,47%)
	C20H18N2O9S	462,44	444 ($M-H_2O$)
	C15H16N2O7S	368,37	367 (M-H,33%)
	C21H20N2O8S	460,47	459 (M-H,31%)
	C17H20N2O8S	412,42	411 (M-H,30%)

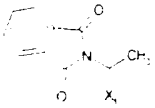
	C17H16N2O7S2	424,45	423 (M-H,16%)
	C20H17N3O7S	443,44	557 (M+TFA, 36%)
	C20H17N3O7S	443,44	442 (M-H,37%)
	C20H17N3O7S	443,44	425 (M-H_2O,23%)
	C19H17N3O7S	431,43	430 (M-H,48%)
	C21H18N2O8S	458,45	414 (M-CO_2,24%)
	C26H24N2O9S	540,55	539 (M-H,17%)
	C21H16N2O9S	472,43	471 (M-H,35%)
	C27H23N3O8S	549,56	663 (M+TFA,36%)
	C18H16N2O7S2	436,47	437 (M+H,45%)
	C20H20N2O7S	432,46	431 (M-H,20%)
	C16H14N2O8S	394,36	393 (M-H,43%)

	C22H18N2O7S	454,46	453 (M-H,42%)
	C19H18N2O8S	434,43	433 (M-H,22%)
	C19H18N2O7S	418,43	417 (M-H,28%)
	C21H22N2O9S	478,48	477 (M-H,25%)
	C21H22N2O8S	462,48	461 (M-H,33%)
	C20H18N2O7S	430,44	429 (M-H,57%)
	C22H22N2O9S	490,49	446 (M-CO_2,42%)
	C16H17N3O8S	411,39	410 (M-H,14%)
	C16H14N2O8S	394,36	393 (M-H, 39%)
	C16H14N2O7S2	410,43	409 (M-H,51%)
	C21H17N3O9S	487,45	486 (M-H,17%)
	C16H14N4O7S	406,38	405 (M-H,17%)
	C17H15N3O8S	421,39	420 (M-H,18%)

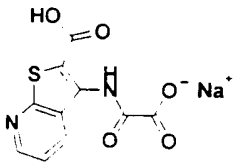
	C17H15N3O7S	405,39	404 (M-H,43%)
	C17H15N3O7S	405,39	404 (M-H,41%)
	C21H16F6N2O7S	554,43	553 (M-H,18%)
	C20H20N2O9S2	496,52	495 (M-H,51%)
	C20H16F2N2O7S	466,42	465 (M-H,43%)
	C16H16N2O8S	396,38	510 (M+TFA,21%)
	C22H19N3O9S	501,48	500 (M-H,23%)
	C18H22N2O8S	426,45	425 (M-H,24%)
	C21H23N3O7S	461,50	460 (M-H,23%)
	C19H16N2O9S	448,41	447 (M-H,42%)
	C22H23N3O8S	489,51	488 (M-H,33%)
	C20H18N2O9S	462,44	418 (M-CO_2,27%)

	C20H18N2O8S	446,44	445 (M-H,16%)
	C20H19N3O8S	461,45	460 (M-H,21%)
	C16H18N2O8S	398,39	380 (M-H_2O,25%)
	C18H16N2O8S	420,40	421 (M+H,39%)
	C15H17N3O8S	399,38	398 (M-H,19%)
	C19H18N2O7S2	450,49	449 (M-H,23%)
	C20H20N2O7S2	464,52	463 (M-H,31%)
	C21H17N3O8S	471,45	470 (M-H,32%)
	C22H19N3O8S	485,48	No hit
	C20H17N3O10S	491,44	No hit
	C22H21N3O8S	487,49	486 (M-H,17%)
	C18H21N3O8S	439,45	438 (M-H,30%)

	C25H21N5O9S	567,54	566 (M-H,32%)
	C23H22N2O10S	518,50	519 (M+H,15%)
	C21H20N4O7S	472,48	471 (M-H,41%)
	C23H21N3O9S	515,50	514 (M-H,45%)
	C16H19N3O8S	413,41	412 (M-H,26%)
	C18H23N3O8S2	473,53	472 (M-H,31%)
	C25H25N3O9S	543,56	542 (M-H,20%)
	C18H23N3O8S	441,46	440 (M-H,28%)
	C28H23N3O9S	577,57	576 (M-H,17%)
	C18H16N2O8S	420,40	419 (M-H,34%)
	C22H22N2O7S	458,49	457 (M-H,22%)
	C26H18N2O9S	534,51	No hit

	C23H20N2O8S	484.49	No hit
	C21H16N2O9S	472.43	471 (M-H,30%)
	C21H18N2O8S	458,45	457 (M-H,27%)
	C22H19N3O9S	501,48	500 (M-H,30%)

EXAMPLE 133



3-(Oxalyl-amino)-thieno[2,3-b]pyridine-2-carboxylic acid, mono sodium salt:

To a stirred solution of 3-amino-thieno[2,3-b]pyridine-2-carboxylic acid methyl ester (1.0 g, 4.8 mmol), triethylamine (1.0 ml, 7.20 mmol) in dry tetrahydrofuran (50 ml) at 0 °C was added dropwise a solution of ethyl oxalyl chloride (0.8 g, 5.76 mmol) in dry tetrahydrofuran (5 ml). The resulting reaction mixture was stirred at room temperature for 3 h. pored into ice water (200 ml). The precipitate was filtered off and dried in vacuo at 50 °C which afforded 0.9 g (61 %) of 3-(ethoxyoxalyl-amino)-thieno[2,3-b]pyridine-2-carboxylic acid methyl ester as a solid.

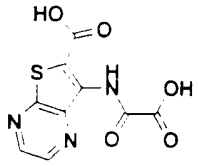
The a solution of the above thieno[2,3-b]pyridine-2-carboxylic acid methyl ester (0.5 g, 1.62 mmol) in ethanol (20 ml) was added a solution of sodium hydroxide (0.2 g, 4.87 mmol) in water (10 ml). The resulting reaction mixture was stirred at room temperature for 18 h., acidified to pH ≈ 4 by addition of 1N aqueous hydrochloric acid the precipitate was filtered off

and wasned with water (2 x 50 ml), diethyl ether (2 x 30 ml) and dried in vacuo at 50 °C affording 130 mg (30 %) of the title compound as a solid.

M.p.: > 250 °C

Calculated for $C_{10}H_5N_2O_5S_1Na_1$, 1 x H_2O ;
C, 39.22 %; H, 2.30 %; N, 9.15 %. Found:
C, 39.32 %; H, 2.35 %; N, 8.89 %.

EXAMPLE 134



7-(Oxalyl-amino)-thieno[2,3-b]pyrazine-6-carboxylic acid;

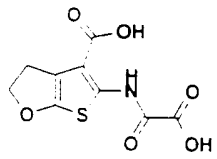
To a solution of 6-amino-thieno[2,3-b]pyrazine-7-carboxylic acid methyl ester (62.7 mg, 0.3 mmol) in tetrahydrofuran (0.5 ml) was added imidazol-1-yl-oxo-acetic acid *tert*-butyl ester (117.6 mg, 0.6 mmol) and triethylamine (42 µl, 0.3 mmol). The resulting mixture was stirred for 20 h at room temperature.
The volatiles were removed in vacuo and the residue was dissolved in ethyl acetate (5.0 ml), washed with 1 % hydrochloric acid (2 x 2 ml), water (2 x 2 ml), dried ($MgSO_4$), filtered and the solvent evaporated in vacuo affording 96 mg (95 %) of 6-(*tert*-butoxyoxalyl-amino)-thieno[2,3-b]pyrazine-7-carboxylic acid methyl ester as a solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 9H), 3.80 (s, 3H), 8.60 (d, 1H, J = 1.5 Hz), 8.70 (d, 1H, J = 1.5 Hz).

To a solution of the above 6-(*tert*-butoxyoxalyl-amino)-thieno[2,3-b]pyrazine-7-carboxylic acid methyl ester (37.8 mg, 0.112 mmol) in dioxane (1.2 ml) was added lithium hydroxide (45 mg) and water (0.6 ml) and the mixture was stirred for 20 h at room temperature. The volatiles were evaporated in vacuo and the residue dissolved in ethyl acetate (30 ml), washed with 1 0 N hydrochloric acid (3 x 3 ml), water (3 x 3 ml), dried (Na_2SO_4), filtered and the solvent evaporated in vacuo affording 20 mg (67 %) of the title compound as a solid.

^{1}H NMR (400 MHz, CD_3OD) δ 8.64 (d, 1H, J = 1.5 Hz), 8.66 (1H, d, J = 1.5 Hz).

MS *m/z* 150.0 (M-117) loose of COOH and COCOOH.

EXAMPLE 135



5-(Oxalyl-amino)-2,3-dihydro-thieno[2,3-b]furan-4-carboxylic acid;

To a solution of dihydro-furan-3-one (11.5 g, 0.134 mol, prepared as described in Org. Syn. Coll. Vol. **5**, 866) in ethanol (200 ml) was added ethyl cyanoacetate (16.6 g, 0.147 mol), sulfur (4.7 g, 0.147 mol) and morpholine (15 ml). The moderate exothermic reaction was stirred at 45 °C for 1 h. The reaction mixture was cooled, filtered and the filtrate evaporated in vacuo. The resultant oil was dissolved in ethyl acetate (400 ml), washed with water (2 x 100 ml), brine (100 ml) and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue (28 g) was subjected to flash column chromatography (1 l silicagel) using ethyl acetate/hexanes (1:1) as eluent. Semi-pure fractions were collected affording after evaporation in vacuo crude 8.4 g of 5-amino-2,3-dihydro-thieno[2,3-b]furan-4-carboxylic acid ethyl ester as an oil.

To the above 5-amino-2,3-dihydro-thieno[2,3-b]furan-4-carboxylic acid ethyl ester (8.4 g, 0.039 mol) dissolved in dry tetrahydrofuran (150 ml), was added triethylamine (10 ml) and a mixture of ethyl oxalyl chloride (4.9 g, 0.043 mol) in dry tetrahydrofuran (25 ml) was added dropwise at 0 °C under nitrogen. The reaction mixture was allowed to stir at room temperature for 18 hours. The volatiles were evaporated in vacuo and the residue dissolved in ethyl acetate (400 ml). The organic phase was washed with water (200 ml), brine (100 ml), dried (Na_2SO_4), filtered and the organic phase evaporated in vacuo. The residue was filtered through a path of silicagel using a mixture of ethyl acetate and heptane (1:1) as eluent. The solvent was evaporated in vacuo and the residue was subjected to flash column chromatography (1 l silicagel) using ethyl acetate/hexanes (1:2) as eluent. Pure fractions were collected affording after evaporation in vacuo and washing of the residue with diethyl ether 0.5 g (1.2

%) of 5-(ethoxyoxalyl-amino)-2,3-dihydro-thieno[2,3-b]furan-4-carboxylic acid ethyl ester as an oil.

To a solution of the above 5-(ethoxyoxalyl-amino)-2,3-dihydro-thieno[2,3-b]furan-4-carboxylic acid ethyl ester (0.4 g, 1.2 mmol) in a mixture of ethanol (10 ml) and water (25 ml) was added a solution of 1 N sodium hydroxide (3.8 ml, 3.8 mmol). The mixture was stirred for 20 h at room temperature. The reaction mixture was diluted with water (50 ml) and washed with ethyl acetate (50 ml). The aqueous phase was acidified with 1 N hydrochloric acid to pH = 2. The precipitate was filtered off and washed with water, dried in vacuo at 50 °C affording 0.2 g of 5-(oxalyl-amino)-2,3-dihydro-thieno[2,3-b]furan-4-carboxylic acid ethyl ester according to NMR. The mono-ester was dissolved in a mixture of water (40 ml) and ethanol (10 ml) and to this mixture was added 1 N sodium hydroxide (3 ml, 3 mmol). The mixture was stirred for 20 h at room temperature. The mixture was acidified with 1 N hydrochloric acid to pH = 2 and the precipitate was filtered off, washed with water and dried in vacuo at 50 °C affording 150 mg (46 %) of the title compound as a solid.

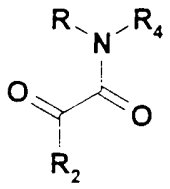
M.p.: > 250 °C.

^{1}H NMR (300 MHz, DMSO-d_6) δ 3.12 (t, 2H), 4.89 (t, 2H), 12.0 (bs, 1H, N*H*CO).

Claims

1. A compound that fulfills all of the following 3 criteria:

(1) has a structure represented by Formula I:



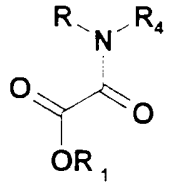
Formula I

where R, R_2 and R_4 are any chemical group or combination of chemical groups;

(2) acts as a phosphotyrosine recognition unit ligand, preferably an inhibitor or modulator of one or more PTPases or proteins that contain SH2 domains; and

(3) has a molecular weight below or equal to 2500 daltons

2. A compound according to claim 1 which has a structure represented by Formula II

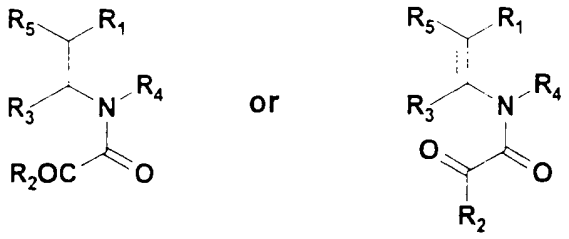


Formula II

where R, R_1 and R_4 are any chemical group or combination of chemical groups, and R_1 preferably is H.

3. A compound that fulfills all of the following 3 criteria:

(1) has a structure represented by Formula III:



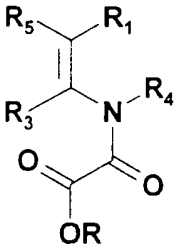
Formula III

where R_1, R_2, R_3, R_4 and R_5 are any chemical group or combination of chemical groups, and R_3 and R_5 may be covalently linked to each other;

(2) acts as a phosphotyrosine recognition unit ligand, preferably an inhibitor or modulator of one or more PTPases or proteins that contain SH2 domains; and

(3) has a molecular weight below or equal to 2500 daltons.

4. A compound according to claim 3 which has a structure represented by Formula IV



Formula IV

where R_1, R_3, R_4 and R_5 are any chemical group or combination of chemical groups, and R_3 and R_5 may be covalently linked to each other, and R preferably is H.

5. A compound according to claim 4 which has a structure represented by Formula V

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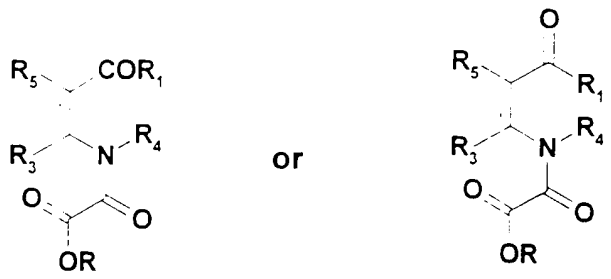
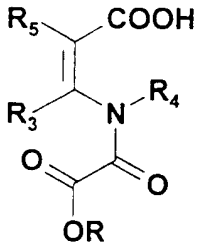


Formula V

where R_1, R_3, R_4 and R_5 are any chemical group or combination of chemical groups, and R_3 and R_5 may be covalently linked to each other, and R is preferably H.

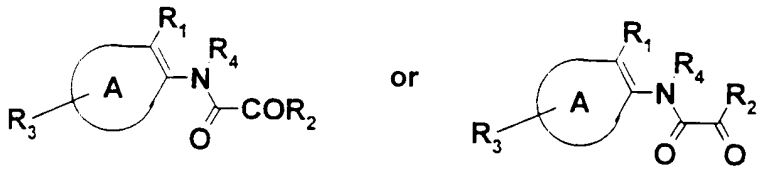
6. A compound according to claim 5 which has a structure represented by Formula VI



Formula VI

where R_3, R_4 and R_5 are any chemical group or combination of chemical groups, and R_3 and R_5 may be covalently linked to each other, and R is preferably H.

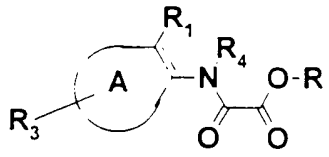
7. A compound according to claim 3, which has a structure represented by Formula VII



Formula VII

where **A** together with the double bond in formula VII represents any aryl as defined above, and R_1, R_2, R_3 and R_4 are any chemical group or combination of chemical groups.

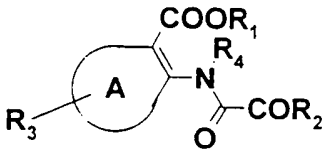
8. A compound according to claim 7, which has a structure represented by Formula VIII



Formula VIII

where A together with the double bond in formula VIII represents any aryl as defined above, and R, R_1, R_3 and R_4 are any chemical group or combination of chemical groups, and R preferably is H.

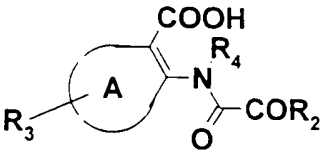
9. A compound according to claim 7 which has a structure represented by Formula IX



Formula IX

where A together with the double bond in formula IX represents any aryl as defined above, and R_1, R_2, R_3 and R_4 are any chemical group or combination of chemical groups.

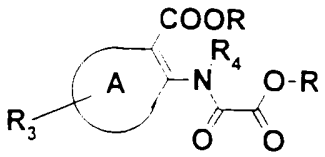
10. A compound according to claim 9 which has a structure represented by Formula X



Formula X

where A together with the double bond in formula X represents any aryl as defined above, and R_2, R_3 and R_4 are any chemical group or combination of chemical groups.

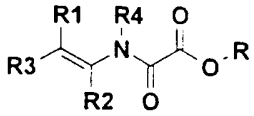
11. A compound according to claim 10 which has a structure represented by Formula XI



Formula XI

where **A** together with the double bond in formula XI represents any aryl as defined above, and R, R_3 and R_4 are any chemical group or combination of chemical groups, and R preferably is H.

12. A compound according to claim 3 which has a structure represented by Formula XII



Formula XII

where R_1 is a chemical group capable of being a proton donor and/or a proton acceptor, preferably -COOH, 5-tetrazolyl, $-NH_2$, $-CONH_2$, and R, R_2, R_3 and R_4 are any chemical group or combination of chemical groups.

13. A compound according to any one of the preceding claims which substantially acts as a classical, competitive inhibitor of one or more PTPases.

14. A compound according to any one of claims 1 to 12 which substantially acts as a mixed-type inhibitor of one or more PTPases.

15. A compound according to any one of claims 1 to 14 which substantially acts as an inhibitor of one or more PTPases involved in regulation of tyrosine kinase signalling pathways.

16. A compound according to any one of claims 1 to 14 which substantially inhibits or modulates receptor-tyrosine kinase signalling pathways via interaction with one or more regulatory PTPases, preferably the signalling pathways of the insulin receptor, the IGF-I receptor and/or other members of the insulin receptor family, the EGF-receptor family, the platelet-derived

growth factor receptor family, the nerve growth factor receptor family, the hepatocyte growth factor receptor family, the growth hormone receptor family and/or members of other receptor-type tyrosine kinase families.

17. A compound according to any one of claims 1 to 14 which substantially inhibits or modulates non-receptor tyrosine kinase signalling through modulation of one or more regulatory PTPases, preferably modulation of members of the Src kinase family or other intracellular kinases.

18. A compound according to any one of claims 1 to 14 which substantially inhibits or modulates the activity of one or more PTPases that negatively regulate signal transduction pathways.

19. A compound according to any one of claims 1 to 14 which inhibits or modulates the activity of one or more PTPases that positively regulate signal transduction pathways, preferably CD45.

20. A compound according to any one of claims 1 to 14 which inhibits or modulates the activity of one or more PTPases that positively regulate signal transduction pathways in immune cells.

21. A compound according to any one of claims 1 to 14 which inhibits or modulates the activity of one or more PTPases that negatively regulate signal transduction pathway.

22. A compound according to any one of claims 1 to 14 which inhibits one or more PTPases via binding to the active site of said PTPase(s) or to other sites that negatively influences the binding of substrate to said PTPase(s), an allosteric modulator.

23. A compound according to any one of claims 1 to 14 which modulates the activity of one or more PTPases via interaction with structures positioned outside of the active sites of the enzymes, preferably SH2 domains.

24. A compound according to any one of claims 1 to 14 which modulates the signal transduction pathways via binding of the compounds of the invention to SH2 domains or PTB domains of non-PTPase signalling molecules.

25. A compound according to any of the preceding claims characterized by being a selective PTPase inhibitor or a compound that is a selective phosphotyrosine recognition unit ligand.

26. A compound according to any of claims 1 to 24 characterized by being a non-selective PTPase inhibitor.

27. A compound according to claim 26 characterized by being an inhibitor or modulator of at least 4 PTPases or 4 PTPase families.

28. A compound according to claim 25 characterized by being selective for a PTPase not described herein.

29. A compound according to claim 25 characterized by being selective for a PTPase listed in Table 1.

30. A compound according to claim 25 characterized by being selective for the PTPα family.

31. A compound according to claim 25 characterized by being selective for PTPα.

32. A compound according to claim 25 characterized by being selective for PTPε.

33. A compound according to claim 25 characterized by being selective for CD45.

34. A compound according to claim 25 characterized by being selective for PTPβ family.

35. A compound according to claim 25 characterized by being selective for PTPβ.

36. A compound according to claim 25 characterized by being selective for PTP-DEP1.

37. A compound according to claim 25 characterized by being selective for PTP-LAR family.

38. A compound according to claim 25 characterized by being selective for PTP-LAR.

39. A compound according to claim 25 characterized by being selective for PTPσ.

40. A compound according to claim 25 characterized by being selective for PTPδ.

41. A compound according to claim 25 characterized by being selective for PTPμ family.

42. A compound according to claim 25 characterized by being selective for PTPμ.

43. A compound according to claim 25 characterized by being selective for PTPκ.

44. A compound according to claim 25 characterized by being selective for PTP1B family.

45. A compound according to claim 25 characterized by being selective for PTP1B.

46. A compound according to claim 25 characterized by being selective for TC-PTP.

47. A compound according to claim 25 characterized by being selective for SHP-PTP family.

48. A compound according to claim 25 characterized by being selective for SHP-1.

49. A compound according to claim 25 characterized by being selective for SHP-2.

50. A compound according to claim 25 characterized by being selective for PTPζ family.

51. A compound according to claim 25 characterized by being selective for PTPζ.

52. A compound according to claim 25 characterized by being selective for PTPγ.

53. A compound according to claim 25 characterized by being selective for PTP-PEST family.

54. A compound according to claim 25 characterized by being selective for PTPH1 family.

55. A compound according to claim 25 characterized by being selective for PTPH1.

56. A compound according to claim 25 characterized by being selective for PTPD1.

57. A compound according to claim 25 characterized by being selective for PTPD2.

58. A compound according to claim 25 characterized by being selective for PTPMEG1.

59. A compound according to claim 25 characterized by being selective for IA-2 family.

60. A compound according to claim 25 characterized by being selective for IA-2.

61. A compound according to claim 25 characterized by being selective for IA-2β.

62. A compound according to claim 25 characterized by being selective for PTPψ family.

63. A compound according to claim 25 characterized by being selective for PTPψ.

64. A compound according to claim 25 characterized by being selective for PTPρ.

65. A compound according to claim 25 characterized by being selective for PTPϕ.

66. A compound according to any one of the preceding claims having a molecular weight of less than 1000 Daltons, and preferably of more than 100 Daltons.

67. A compound according to any one of the preceding claims having a K_i value of less than 200 μM against one or more PTPases.

68. A compound according to any one of the preceding claims having a K_i value of less than 2 μM against one or more PTPases.

69. A compound according to any one of the preceding claims having a K_i value of less than 100 nM against one or more PTPases.

70. A compound according to any one of the preceding claims having a IC_{50} value of less than 200 M against one or more molecules with phosphotyrosine recognition unit(s).

71. A compound according to any one of the preceding claims having a IC_{50} value of less than 2 M against one or more molecules with phosphotyrosine recognition unit(s).

72. A compound according to any one of the preceding claims having a IC_{50} value of less than 100 nM against one or more molecules with phosphotyrosine recognition unit(s).

73. A compound according to any one of claims 1 to 66 having a K_i value of < 2 μM against one or two PTPases or PTPase families and a K_i value of > 50 μM against at least two other PTPases or PTPase families.

74. A compound according to any one of claims 1 to 66 having a K_i value of < 100 nM against one or two PTPases or PTPase families and a K_i value of > 10 μM against at least two other PTPases or PTPase families.

75. The use of a compound according to any one of the preceding claims for preparing a medicament for modulating the activity of one or more PTPases or other molecules with phosphotyrosine recognition unit(s).

76. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing type I diabetes, type II diabetes, impaired glucose tolerance, insulin resistance, obesity, immune dysfunctions including autoimmunity and AIDS, diseases with dysfunctions of the coagulation system, allergic diseases, osteoporosis, proliferative disorders including cancer and psoriasis, diseases with decreased or increased synthesis or effects of growth hormone, diseases with decreased or increased synthesis of hormones or cytokines that regulate the release of/or response to growth hormone, diseases of the brain including Alzheimer's disease and schizophrenia, and infectious diseases.

77. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing type I diabetes, type II diabetes, impaired glucose tolerance, insulin resistance, and/or obesity.

78. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing conditions with immune dysfunctions, including autoimmunity such as rheumatoid arthritis, systemic lupus erythematosus.

79. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for use as an immunosuppressant.

80. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing or treating conditions with immune dysfunctions including AIDS.

81. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing allergic diseases, including asthma and allergic skin diseases.

82. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing proliferative disorders, including cancer.

83. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing osteoporosis.

84. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing psoriasis.

85. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing diseases with decreased or increased synthesis or effects of growth hormone, diseases with decreased or increased synthesis of hormones or cytokines that regulate the release of/or response to growth hormone.

86. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing diseases with dysfunctions of the coagulation system.

87. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing diseases of the brain including Alzheimer's disease and schizophrenia.

88. The use of a compound according to any one of claims 1 to 74 for preparing a medicament for managing, treating or preventing infectious diseases.

89. A pharmaceutical composition comprising an effective amount of a compound according to any one of claims 1 to 74 together with a pharmaceutically acceptable carrier or diluent.

90. The pharmaceutical composition according to claim 89 comprising between 0.5 mg and 1000 mg of a compound according to any one of the claims 1 to 74 per unit dose.

91. A method of modulating the activity of one or more PTPases or other molecules with phosphotyrosine recognition unit(s) in a subject in need of such management comprising administering to said subject an effective amount of a compound or composition according to any one of claims 1 to 74.

92. A compound according to any one of claims 1 to 74 coupled to a suitable solid-phase matrix.

93. A method for isolating a protein or a glycoprotein with affinity for a compound according to any one of claims 1 to 74 from a biological sample, comprising:

- contacting an immobilized compound according to claim 89 with said biological sample in order for said immobilized compound to form a complex by binding said protein or glycoprotein,
- removing unbound material from said biological sample and isolating said complex, and
- extracting said protein or glycoprotein from said complex.

94. A method for isolating a protein-tyrosine phosphatase with affinity for a compound according to any one of claims 1 to 71 from a biological sample, comprising

- contacting an immobilised compound according to claim 89 with said biological sample in order for said immobilised compound to form a complex by binding said protein-tyrosine phosphatase
- removing unbound material from said biological sample and isolating said complex
- extracting said protein-tyrosine phosphatase.

95. A method for isolating a Src-homology 2 domain containing protein or a phosphotyrosine binding domain containing protein with affinity for a compound according to any one of the preceding compound claims from a biological sample, comprising

- contacting an immobilized compound according to claim 89 with said biological sample in order for said immobilized compound to form a complex by binding said Src-homology 2 domain containing protein or a phosphotyrosine binding domain containing protein
- removing unbound material from said biological sample and isolating said complex
- extracting said Src-homology 2 domain containing protein or a phosphotyrosine binding domain containing protein from said complex.

96. A compound according to any one of claims 1 to 71 coupled to a fluorescent or radioactive molecule.

97. A method for coupling a fluorescent or radioactive molecule to a compound according to any one of claims 1 to 71 comprising

- contacting said compound with said fluorescent or radioactive molecule in a reaction mixture to produce a complex

- removing uncomplexed material and isolating said complex from said reaction mixture.

98. A method for detecting protein-tyrosine phosphatase or other molecules with phosphotyrosine recognition unit(s) in a cell or in a subject using a compound according to claim 93 comprising

- contacting said cell or an extract thereof or a biological sample from said subject or by injecting said compound into said subject in order for said compound to produce a complex with said protein-tyrosine phosphatase or said molecules with phosphotyrosine recognition unit(s)
- detecting said complex, thereby detecting the presence of said protein tyrosine phosphatase or said other molecules with phosphotyrosine recognition unit(s).

99. A method for quantifying the amount of protein-tyrosine phosphatases or other molecules with phosphotyrosine recognition unit(s) in a cell or in a subject using a compound according to claim 93 comprising

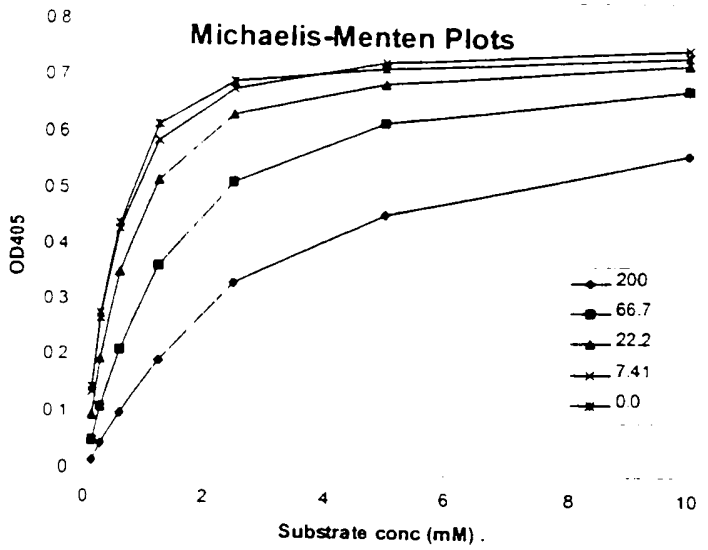
- contacting said cell or an extract thereof or a biological sample from said subject or by injecting said compound into said subject in order for said compound to produce a complex with said protein-tyrosine phosphatase or said molecules with phosphotyrosine recognition unit(s)
- measuring the amount of said complex, thereby detecting the presence of said protein tyrosine phosphatase or said molecules with phosphotyrosine recognition unit(s).

100. A method for determining the function of a given protein-tyrosine phosphatase or group of protein-tyrosine phosphatases or said molecules with phosphotyrosine recognition unit(s) in a cell or a subject using a compound according to claim 93 comprising

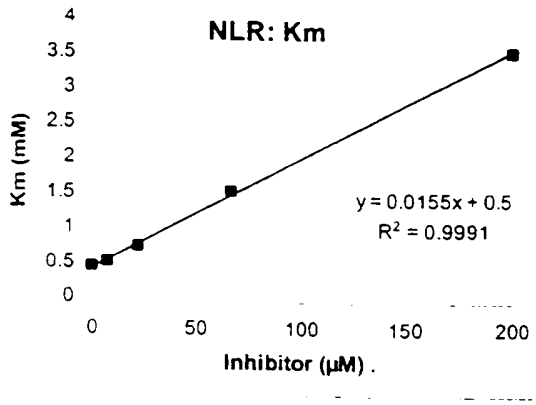
- contacting said cell or an extract thereof or a biological sample from said subject or by injecting said compound into said subject in order for said compound to produce a complex with said protein-tyrosine phosphatase or said molecules with phosphotyrosine recognition unit(s)
- measuring the biological effects induced by said complex.

101. A compound which after uptake in cells or mammals has a structure and a function as defined in any one of claims 1 to 71.

(A)



(B)



(C)

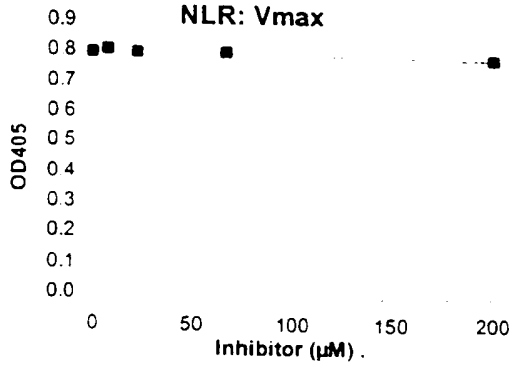
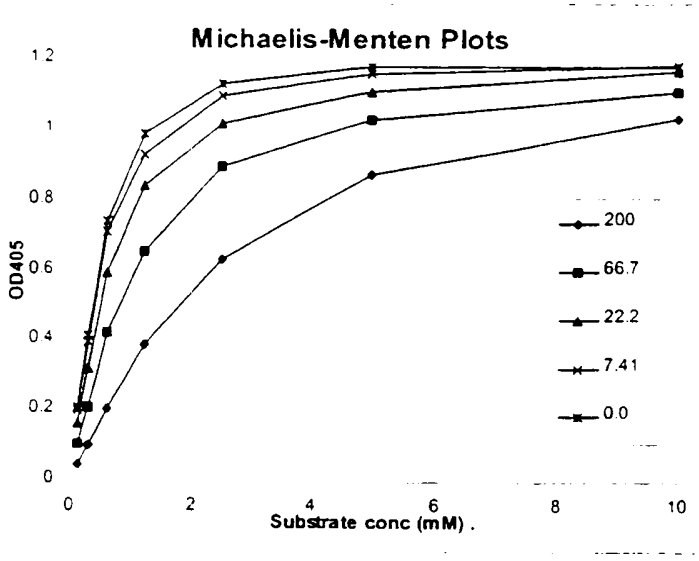
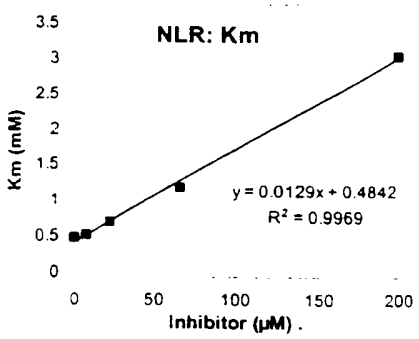


Figure 1
(exp. 1230-5)

(A)



(B)



(C)

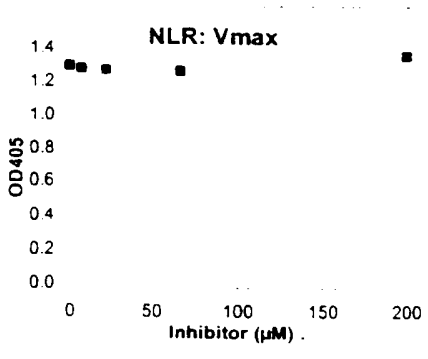
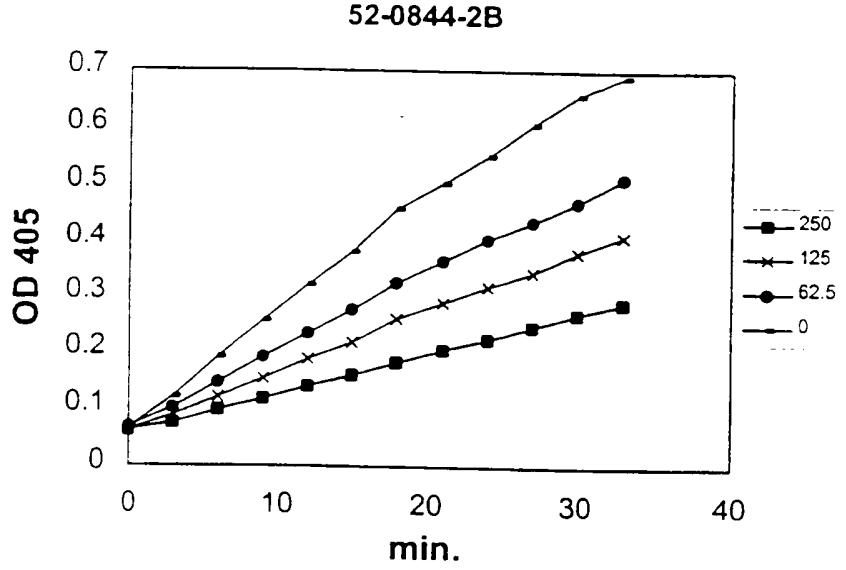


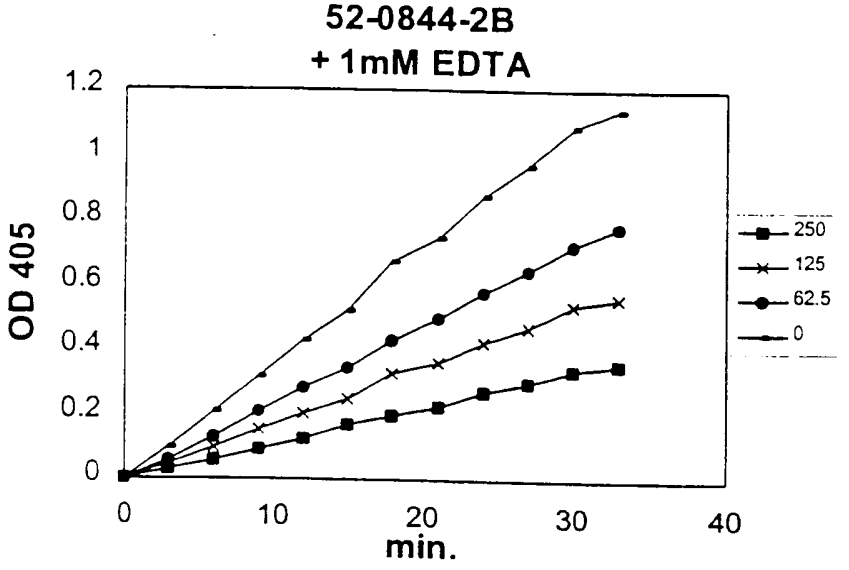
Figure 2
(exp. no. 1167-3)

(A)



Time course exp. 172

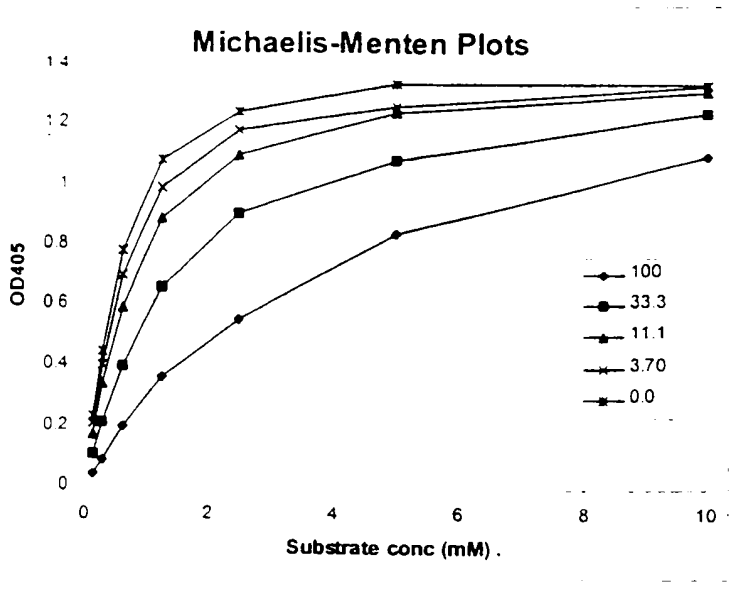
(B)



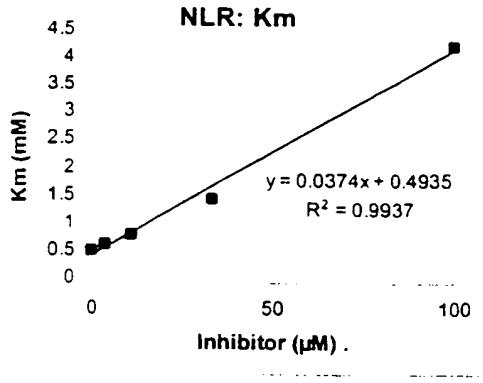
Time course exp. 173

Figure 3

(A)



(B)



(C)

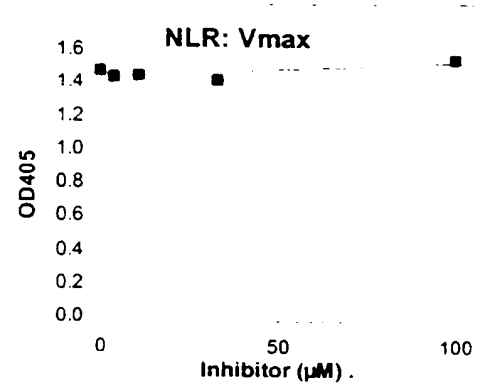
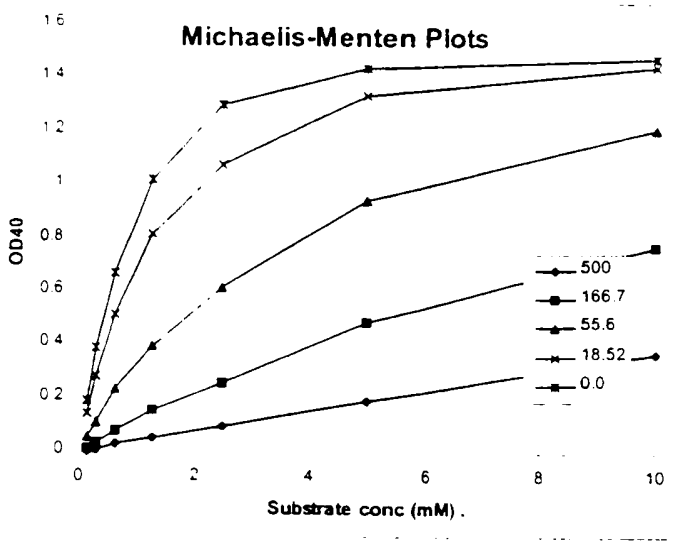


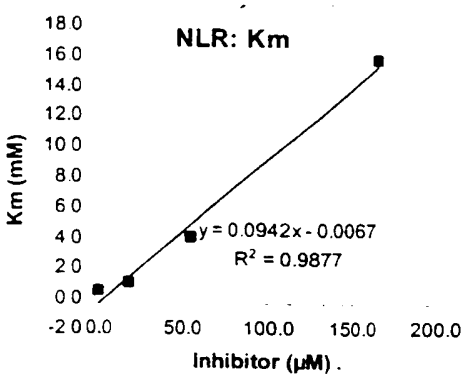
Figure 4

Ex.p. no. 1251-10

(A)



(B)



(C)

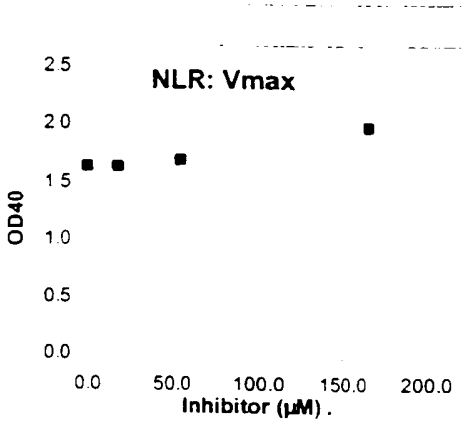


Figure 5
Exp. no. 1264-6

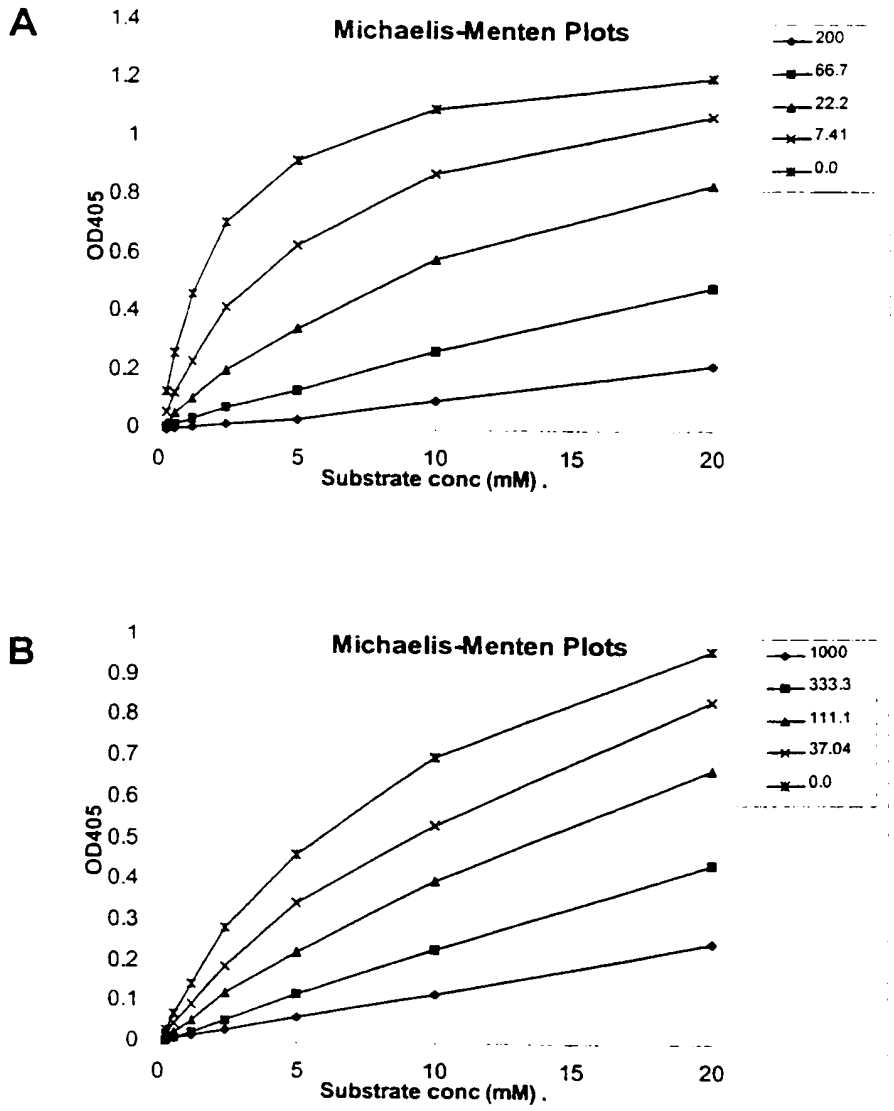
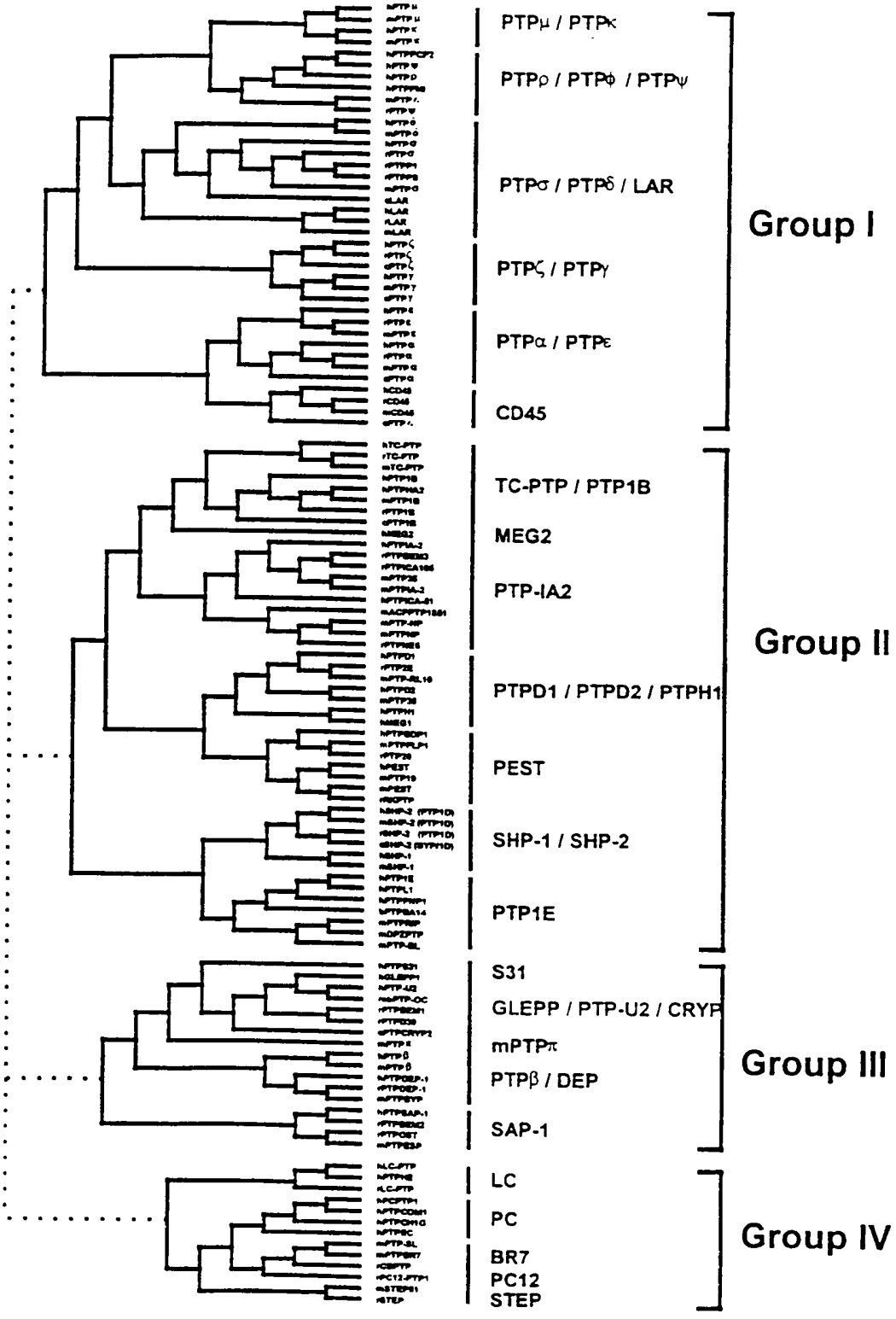


Figure 6

Figure 7. Homology Tree Based on Multiple Sequence Alignments of PTPase Domains



INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00126

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07C 235/84, C07D 213/55, C07D 239/28, C07D 241/24, C07D 513/04, C07D 333/62, C07D 497/04, C07D 209/18, A61K 31/17, 31/44, 31/50, 31/505, 31/38, 31/395
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07D, C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9640113 A2 (SUGEN, INC.), 19 December 1996 (19.12.96), Compound 22 --	1
X	Cancer research, Volume 52, No 16, 1992, Johanna F. Geissler et al, "Benzopyranones and Benzothiopyranones: A Class of Tyrosine Protein Kinase Inhibitors with Selectivity for the v-abl Kinase" page 4492 - page 4498 -- --------	1

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 1999	13 -07- 1999
Name and mailing address of the ISA Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Göran Karlsson/Els Telephone No. + 46 8 782 25 00

Form PCT/ISA 210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK99/00126

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1 ☒ Claims Nos. 91
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy, see rule 39.1

2 ☒ Claims Nos.: 1-90, 92-98
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next page

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1 ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2 ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3 ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4 ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

The present claims are drafted in such a complex and broad way that no meaningful search is possible to perform, cf. Article 6. The multitude of variables and their permutations and combinations result in a claimed subject matter that is so broad in scope that it is rendered virtually incomprehensible.

A preliminary search of a part of claim 1 has shown that a great number of known compounds are included in formula I in this claim. The only meaningful search has been limited to compounds which are in the databases and are given as fulfilling characteristic (2). However as the characteristic (2) is not always mentioned in the databases and thus not reliably possible use for a search for novelty, the search is only preliminary. Such functional characteristics are only allowed when possible to use for search and examination. This is not the case here.

The claimed subject matter lacks a significant structural element qualifying as a special technical feature that clearly defines a contribution over the prior art. Due to the lack of such a feature and the complexity of the claims, the present claims state a great number of separate inventions. No invitation to pay additional fees has been made as this would have meant a unreasonable number of fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/06/99

International application No.
PCT/DK 99/00126

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9640113 A2	19/12/96	AU 6110496 A	30/12/96
		EP 0831795 A	01/04/98

Form PCT/ISA/210 (patent family annex) (July 1992)